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NOTICE

The following courses of instruction will be given by the Liverpool School of Tropical Medicine during 1911:—

Full Course begins 6 January. Short Course begins 1 June.
Diploma Examination, 3 April. Certificate Examination, 29 June.
Full Course begins 15 September.
Diploma Examination, 11 December.

The full Course of Instruction is open to all qualified medical men, and the Examination to all students who have taken out this full course.

Fee for the full Course of Instruction—Thirteen Guineas.

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Fee for the Short Course of Instruction—Four Guineas.

Fee for the use of a School microscope during one term—Ten shillings and sixpence.

For prospectus and further information, application should be made to the Dean of the Medical Faculty, University of Liverpool.

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1904	Bennett, Arthur King
1904	Bruce, William James
1904	Byrne, John Scott
1904	Clayton, Thomas Morrison
1904	Dalziel, John McEwen
1904	Dee, Peter
1904	Greenidge, Oliver Campbell
1904	Hehir, Patrick
1904	Khan, Saiduzzafor
1904	Laurie, Robert
1904	Maclurkin, Alfred Robert
1904	McConnell, Robert Ernest
1904	Nicholson, James Edward
1904	Philipson, Nicholas
1904	Sharman, Eric Harding
1904	Thomson, Frank Wyville
1904	Walker, George Francis Clegg
1905	Anderson, Catherine Elmslie
1905	Brown, Alexander
1905	Caldwell, Thomas Cathcart
1905	Critien, Attilio
1905	Hooton, Alfred
1905	Hudson, Charles Tilson
1905	Illington, Edmund Moritz

Date of Diploma	
1905	Macfarlane, Robert Maxwell
1905	Maddock, Edward Cecil Gordon
1905	Moore, James Jackson
1905	Nightingale, Samuel Shore
1905	Radcliffe, Percy Alexander Hurst
1905	Young, John Cameron
1906	Adie, Joseph Rosamond
1906	Arnold, Frank Arthur
1906	Bate, John Brabant
1906	Bennetts, Harold Graves
1906	Carter, Robert Markham
1906	Chisholm, James Alexander
1906	Clements, Robert William
1906	Dundas, James
1906	Faichnie, Norman
1906	Jeffreys, Herbert Castelman
1906	Mackenzie, Donald Francis
1906	Pailthorpe, Mary Elizabeth
1906	Palmer, Harold Thornbury
1906	Pearse, Albert
1906	Sampey, Alexander William
1906	Smithson, Arthur Ernest
1906	Taylor, Joseph van Someron
1906	Taylor, William Irwin
1906	Tynan, Edward Joseph

Date of Diploma	
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1906	Willcocks, Roger Durant
1906	Williamson, George Alexander
1907	Allan, Alexander Smith
1907	Allwood, James Aldred
1907	Bond, Ashton
1907	Branch, Stanley
1907	Collinson, Walter Julius
1907	Davey, John Bernard
1907	Donaldson, Anson Scott
1907	Fell, Matthew Henry Gregson
1907	Gann, Thomas William Francis
1907	Graham, James Drummond
1907	Hiscock, Robert Carroll
1907	Keane, Joseph Gerald
1907	Kennan, Richard Henry
1907	Kenrick, William Hamilton
1907	Le Fanu, George Ernest Hugh
1907	Mackey, Charles
1907	Maddox, Ralph Henry
1907	McCarthy, John McDonald
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1907	Ryan, Joseph Charles
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1908	Caverhill, Austin Mack
1908	Crawford, Gilbert Stewart
1908	Dalal, Kaikhusroo Rustomji
1908	Dansey-Browning, George
1908	Davidson, James
1908	Dickson, John Rhodes
1908	Dowdall, Arthur Melville
1908	Glover, Henry Joseph
1908	Greaves, Francis Wood
1908	Goodbody, Cecil Maurice
1908	Harrison, James Herbert Hugh
1908	Joshi, Lemuel Lucas
1908	Le Fanu, Cecil Vivian
1908	Luethgen, Carl Wilhelm Ludwig
1908	Mama, Jamshed Byramji
1908	McCay, Frederick William
1908	McLellan, Samuel Wilson
1908	Pearce, Charles Ross
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1909	Henry, Sydney Alexander
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1909	Jackson, Arthur Frame
1909	Kaka, Sorabji Manekji
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1909	Samuel, Mysore Gnanandandaraju
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1909	Thornely, Michael Harris
1909	Turkhud, Violet Ackroyd
1909	Webb, William Spinks
1909	Yen, Fu-Chun
1910	Brabazon, Edward
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1910	Caulcrick, James Akilade
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EDITORIAL NOTICE

By order of the Committee of the Incorporated Liverpool School of Tropical Medicine, the series of the Reports of the School, which had been issued since 1899, were followed, from January 1, 1907, by the Annals of Tropical Medicine and Parasitology, of which this is the fourth number of the fourth volume.

Altogether twenty-one Memoirs, besides other works, were published by the School since 1899, and of these ten, containing 519 quarto or octavo pages and 95 plates and figures, were published during the two years 1904 and 1905.

The Annals are issued by the Committee of the School, and will contain all such matter as was formerly printed in the Reports—that is to say, accounts of the various expeditions of the School and of the scientific work done in its laboratories at the University of Liverpool and at Runcorn. In addition, however, to School work, original articles from outside on any subject connected with Tropical Medicine or Hygiene may be published if found suitable (see notice on back of cover); so that, in all probability, not less than four numbers of the Annals will be issued annually. Each number will be brought out when material sufficient for it has been accumulated.

A NOTE ON THE PATHOLOGY OF LESIONS OF THE CORNEA AND SKIN IN ANIMALS EXPERIMENTALLY INFECTED WITH *T. RHODESIENSE*

BY

WARRINGTON YORKE, M.D.

(From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine).

(Received for publication 2 November, 1910)

As mentioned in a previous paper* three goats and a horse infected with *T. rhodesiense* all developed an interstitial keratitis of a remarkably transient character.

GOAT 1. On the sixth day after inoculation the temperature rose to 107° F. and parasites appeared in the peripheral blood, one being found to a field (Zeiss objective DD, eye-piece 4). During the next five days parasites were present in the blood in small numbers, one to from twenty to fifty fields. Subsequently the temperature fell to 103° F., and except on the thirty-first day, when one trypanosome was seen in fifty fields, parasites were not again found in the blood until the day of death, which occurred on the fifty-fifth day after inoculation, when one trypanosome was present in fifty fields.

On the fifteenth day a distinct swelling of the skin and subcutaneous tissue was visible below the eyes and over the nasal bones. This condition became gradually more marked and persisted until the animal's death.

On the seventeenth day it was noticed that the lower portion of the left cornea was slightly milky in appearance. This cloudiness rapidly became more dense, until on the nineteenth day the whole of the lower two-thirds of the left cornea was densely opaque.

* Yorke. 'On the Pathogenicity of a Trypanosome (*T. rhodesiense*) from a case of Sleeping Sickness contracted in Rhodesia,' Ann. Trop. Med. and Parasitol., Vol. IV, p. 351, 1910.

Slight opacity of the right cornea was now apparent. On the twenty-fourth day both corneae were opaque. During the next week the opacity gradually disappeared, until on the thirty-second day the eyes appeared perfectly normal. On the thirty-seventh day the left cornea again became cloudy, and on the thirty-ninth was opaque. The following day slight opacity was observable in the other cornea. The left cornea remained densely opaque and the right slightly cloudy until the death of the animal.

GOAT 2. Parasites were first observed in the blood (one to fifty fields) on the fourth day after inoculation. In the course of the next thirty-three days they were found on ten occasions in very small numbers. During the last nine days no parasites were found. The animal died on the forty-sixth day. The temperature never rose above 104° F. Oedematous swelling of the integuments below the eyes and over the nasal bones appeared on the sixteenth day and persisted until death.

On the thirty-sixth day slight opacity of the left cornea was observed for the first time. The opacity rapidly spread from below upwards, until on the thirty-eighth day the left cornea was densely opaque in its lower two-thirds. On the thirty-ninth day the left cornea was found to be clearing, whilst there was a slight cloudiness of the cornea of the opposite eye. The following day the right cornea was opaque. During the next few days the opacity rapidly disappeared from both eyes, until on the forty-second day the left cornea was clear and the right almost clear. During the last four days the right cornea became slightly more cloudy; the left remained clear until death.

GOAT 3. Parasites (one to fifty fields) first seen in the blood on the eleventh day after inoculation. They were subsequently found in very small numbers on the twenty-first and thirty-ninth days. The animal died on the fifty-fourth day. Temperature remained between 103° and 104° F. Oedematous condition of the skin of the face apparent on the thirtieth day and persisted until death.

Slight cloudiness of the lower portion of the right cornea appeared for the first time on the thirty-ninth day. The opacity rapidly increased in extent and density, until on the forty-second day the whole cornea was densely opaque. The condition persisted until death. The left cornea was unaffected.

In none of the goats was any other pathological condition of the eye observed. The pupils dilated normally with atropine. There was no conjunctivitis nor injection of the sclerotic.

HORSE 1. Parasites were first found in the blood on the seventh day. Subsequently they were present regularly in small numbers until the animal's death on the thirty-eighth day.

Slight haziness of the left cornea was first apparent on the thirty-fifth day. The condition gradually increased in severity, until at the time of death the whole cornea was cloudy.

The right cornea was unaffected. The sclerotic of the left eye was slightly injected.

For the purpose of studying the histological appearances the eyes were removed at the time of death and small portions of the cornea fixed in various fluids, viz., formalin 10 per cent., Flemming's solution and mercuric chloride alcohol. The tissues were embedded in paraffin and the sections stained with Heidenhain's iron alum haematoxylin, Breinl's safranine methylene-blue orange tannin, Giemsa and van Gieson. The best results, so far as the staining of the parasites was concerned, were obtained by staining the tissue which had been fixed in Flemming's solution by either the iron alum haematoxylin or the safranine methylene-blue orange tannin method. In well-fixed sections stained by these methods the trypanosomes stained exceedingly well, and the different portions of the parasites were easily distinguished. On the other hand, in those which had been fixed with formalin, the parasites did not stain, or at the best were very indistinct.

For purposes of description the eight corneae examined may be grouped into four classes.

I. Those which were densely opaque at the time of death, viz., left cornea of Goat 1 and the right of Goat 3. In these the morbid changes were very marked. The pathological conditions in the two were somewhat different. In the left cornea of Goat 1 the lesions were entirely confined to the substantia propria, the anterior and posterior epithelial layers being unaffected. Scattered throughout the substantia propria, which was almost twice as thick as that of a normal eye, were small oedematous patches. These were situated for the most part in the anterior third of the substantia propria. In the distended interlamellar spaces were a few leucocytes (both

polymorphonuclear and mononuclear) and also large numbers of trypanosomes. In the deeper layers of the substantia propria there was marked cellular infiltration and also considerable vascular formation. These areas also extended in places into the anterior third reaching occasionally almost to the anterior homogeneous membrane. Trypanosomes were found scattered throughout this portion of the cornea and in certain spots they existed in enormous numbers.

Appearances somewhat similar to the above have been described by Morax* as occurring in the corneae of dogs and goats infected with *T. equiperdum*.

The state of affairs obtaining in the right cornea of Goat 3 represented a rather more advanced stage. The substantia propria was about three times as thick as that of a normal cornea, and exhibited similar changes to those described in the previous case, viz., markedly oedematous areas lying side by side with patches of intense leucocytic infiltration. There was also some extravasation of red cells from the newly-formed vessels. The morbid processes were not, however, confined to the substantia propria, but had involved the anterior epithelial layer, which in places was separated from the substantia propria by inflammatory exudation. In these regions the epithelial cells were degenerated and stained badly, and at some points were completely disintegrated.

In contradistinction to the previous case only few trypanosomes were found scattered throughout the substantia propria.

II. Those which were slightly opaque at the time of death, viz., right cornea of Goat 1 and of Goat 2 and the left of the horse. Here the lesions were limited to the substantia propria which was slightly increased in thickness. In the deeper layers near the corneo-sclerotic junction there was considerable cellular infiltration and slight vascular formation. Elsewhere the only change visible was a slightly oedematous condition with the occurrence of a few leucocytes and trypanosomes scattered throughout the interlamellar spaces.

III. The left cornea of Goat 2, which after being densely opaque had subsequently become perfectly clear again. The

* 'Manifestations oculaires au cours des Trypanosomiases,' Annales de l'Institut Pasteur, 1907, p. 47.

anterior and posterior epithelial layers were normal. The substantia propria was slightly thicker than normal and oedematous in places. Near the corneo-sclerotic junction there was considerable infiltration of polymorpho- and mono-nuclear leucocytes and some new vascular formation, but elsewhere only a few leucocytes were found. No parasites were seen.

IV. The left cornea of Goat 3 and the right of the horse, which had remained unaffected throughout the disease. In these nothing abnormal was observed microscopically.

A comparable condition of affairs was found to exist in the lesions of the skin of an infected rabbit.

Small pieces of skin were removed from the following different areas:—(*a*) Normal skin of back; (*b*) area recently affected in which the hair was being shed and the skin oedematous; (*c*) bald markedly oedematous area; (*d*) region which had been bald for some weeks and showed some signs of recovery. The thickening of the skin had largely disappeared and there was a slight re-growth of hair.

The methods of fixing and staining were those employed for the cornea.

The histological appearances presented by sections of skin from these different areas may be classified as follows:—

(*a*) No pathological changes and no parasites were found.

(*b*) Large numbers of trypanosomes were found lying in the oedematous interstitial spaces. There was also considerable cellular infiltration.

(*c*) In this region the skin was considerably thickened and markedly oedematous. Enormous numbers of trypanosomes were present. There was great cellular infiltration involving the hair follicles and glands. Many of the hair follicles had completely disappeared.

(*d*) Here no parasites were found. The skin was not distended with fluid and the cellular infiltration was only slight.

Judging from the appearance presented by this series of sections of the cornea and skin the course of events would seem to be somewhat as follows:—

In early lesions trypanosomes are present in the tissues, and as a result there is an oedematous condition of the part, and a more or less marked degree of leucocytic infiltration.

Later as the number of parasites increases the morbid condition becomes more accentuated. Large numbers of leucocytes are poured into the tissue and new vessels develop. After a time the parasites disappear and with their disappearance there is a tendency on the part of the tissue to recovery.

The rapidity with which these processes occurred in the cornea is very remarkable. One can hardly suppose that the changes in the right cornea of Goat 1 and the left of Goat 2, which from being densely opaque were after the interval of a few days apparently normal, had progressed so far as the formation of new vessels; yet, on account of the extreme opacity, there must have been very considerable cellular infiltration, although this might in part be explained by the presence of enormous numbers of parasites such as were seen in the left cornea of Goat 1, which was densely opaque at the time of death.

The fact that trypanosomes can multiply so readily in the tissue spaces and at the same time be either entirely absent from the blood, or present in very small numbers only, is one of considerable interest, although the explanation is not very obvious. Perhaps the tissue juices form a more favourable nidus for the growth of the parasites, or possibly in these situations they escape to some extent the action of certain anti-bodies which have been shown to exist in the blood. Whatever the cause may be, the observation illustrates in what manner it is possible for an animal to be heavily infected and at the same time present no parasites in the peripheral circulation.

The drawings were done by Miss A. M. Brookfield.

DESCRIPTION OF PLATES.

PLATE XXV.

Left cornea of Goat 1.—Fixed with Flemming's solution. Stained by Heidenhain's iron alum haematoxylin method. Drawn with Abbé camera lucida, 2 mm. apochromatic objective and No. 8 compensating ocular (Zeiss). Magnification 1,000 diameters.

Considerable oedematous infiltration of the substantia propria. In the distended interstitial spaces are large numbers of trypanosomes. The parasites are cut in various directions, some transversely and others obliquely, whilst others are lying horizontally in the plane of the section.

There is also considerable infiltration of polymorphonuclear and mononuclear leucocytes. Two small blood vessels are seen in the lower portion of the drawing.

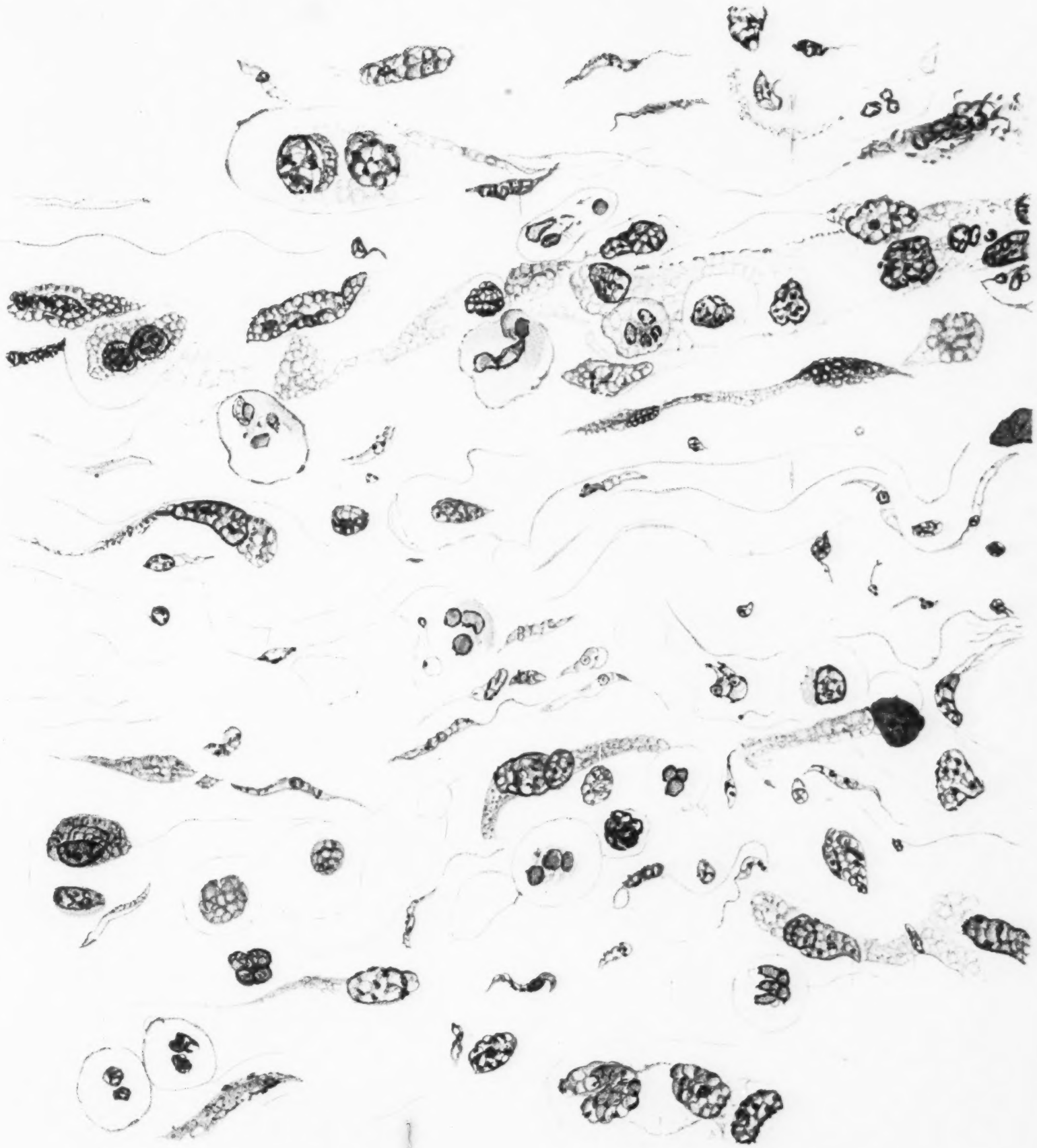
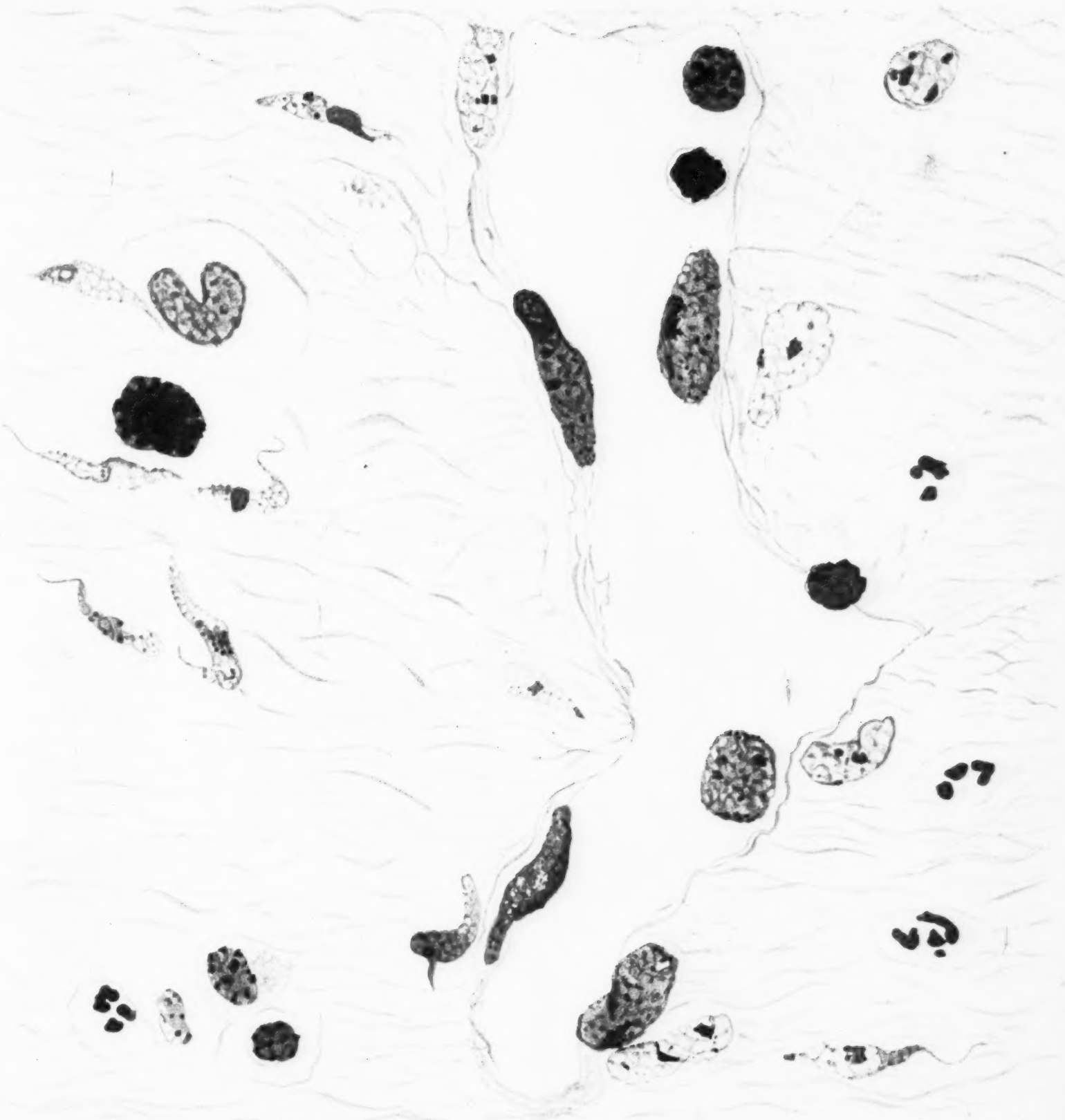


Plate XXVI.

Right cornea of Goat 3.—Fixed with Flemming's solution. Stained by Breinl's safranine methylene-blue orange tannin method. Drawn with Abbé camera lucida, 2 mm. apochromatic objective and No. 12 compensating ocular (Zeiss). Magnification 1,500 diameters.

The drawing represents a small portion of an area of marked cellular infiltration and vascularisation. A small vessel runs vertically across the field. Trypanosomes, polymorpho- and mono-nuclear leucocytes are seen lying between the fibres of the substantia propria.



A CASE OF SLEEPING SICKNESS STUDIED BY PRECISE ENUMERATIVE METHODS: FURTHER OBSERVATIONS

BY

MAJOR RONALD ROSS, F.R.S.,

AND

DAVID THOMSON, M.B., CH.B., D.P.H.

(*Received for publication* 15 *October*, 1910)*

I. INTRODUCTION

In a previous paper† by us, we recorded our observations on this case during two and a half months, and described particularly the regular periodical rises in the numbers of trypanosomes disclosed in the patient's peripheral blood by methodical daily countings extending over that period. Our technique has been detailed in another paper by us on 'Enumerative Studies on Malarial Fever.'‡ We now record our further observations on the case during two more months—until the patient's death. A chart and a table giving daily details of the observations are attached; and accompanying papers by Drs. H. B. Fantham and J. G. Thomson record studies on animals and on the parasites themselves. We must refer also to a recent communication by Drs. J. W. W. Stephens and Fantham§ suggesting that the species found in this case may not be identical with *T. gambiense*.

The patient, a strong young Englishman, age 26, weight 154 lbs., was infected in N.E. Rhodesia near the River Luangwa in September, 1909. The trypanosomes were found in his blood in Africa on November 17.

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* Reprinted from Proc. Roy. Soc., B, Vol. LXXXIII, p. 187.

† Proc. Roy. Soc., July 21, 1910, B, 557.

‡ Ann. Trop. Med. and Parasit., Vol. IV., No. 3.

§ *Ibid*.

He was admitted into the Royal Southern Hospital on December 4. Daily estimation of the number of trypanosomes per cubic millimetre of his blood was commenced on February 16, and continued till his death on June 29, a period of 134 days. During that period we never failed to find trypanosomes. The history of the case from February 16 is recorded graphically on the chart.

The patient had glandular swellings and ill-defined erythematous rashes on his legs at various intervals. On April 20 he had a severe attack of vomiting which continued for four days; in consequence he lost 10 lbs. in weight and was never again so well. He remained in bed onwards till his death. He became progressively more drowsy, with intervals of more or less comparative brightness. His memory and mental powers failed steadily. On May 7 he developed a marked neuritis due to the atoxyl, and every dose seemed to aggravate the neuro-retinitis produced by the previous administration of this drug. His eyes were examined weekly by Dr. Hamilton, oculist to the hospital.

On May 10 both legs became markedly oedematous and remained so till his death. On May 13 he became much worse and developed paralysis of his sphincters. He seemed about to die, but on May 20 a sudden remarkable improvement took place. Soon, however, he again commenced to grow steadily worse.

On June 25 he developed pleurisy and pneumonia. He died on June 29.

Post mortem.—Performed twenty-two hours after death:—

Brain.—Membranes thickened and white. Vessels congested. Cerebrospinal fluid increased.

Left lung.—Broncho-pneumonia.

Left pleural cavity.—Contained one pint of yellow pussy fluid.

Spleen.—Soft and much enlarged; weight, 33 ozs.

Liver.—Enlarged; weight, 6 lbs.

Blood.—No trypanosomes could be found. Leucocytes, 17,000 per cubic millimetre. Polymorph excess.

Smears of bone marrow, spleen, kidney, mesenteric glands, and cerebrospinal fluid, all showed large numbers of small and large mononuclear cells, but no trypanosomes could be detected.

II. CONTINUANCE OF THE PERIODICAL CYCLE

The cycle recorded by us till April 30 in our previous paper continued till his death. From February 22 till June 25 there occurred nineteen rises in the numbers of trypanosomes in his peripheral blood. This gives an average of six and a half days between the heights of each rise.

The shortest period between two successive heights is four days, as recorded between April 18 and 22, and again between June 3 and 7. This exceptionally short interval was due, we think, to artificial means, as we shall explain later under vaccine treatment. The longest period recorded between two successive heights is eight days, while the usual period between the heights seems to be seven days. It will be observed that the rise and fall is sudden, and that the height is not maintained.

While the number of the parasites was increasing rapidly, we observed many forms with double nuclei and blepharoplasts (dividing forms). During the interval between the rises, few of these forms were present. This would seem to indicate that the sudden rise is due to an active multiplication of the parasites.

On April 28 the numbers increased from 200 to 1,536 in twenty-four hours, and on June 19 they increased from 100 to 1,500 in twenty-four hours, suggesting that in our patient they were capable of dividing and sub-dividing three to four times in twenty-four hours.

The natural fall is even more sudden than the rise. On April 29 the parasites fell from 1,536 to 100 per cubic millimetre of blood in twenty-four hours.

Except on April 5 and 6 only one observation was made daily, so that obviously we may have missed the highest points reached in the various rises. The low levels were, as a rule, maintained for three or four days, forming **U**-shaped bends as recorded in the chart. The lowest number recorded was 8 per cubic millimetre of blood, while the highest reached 1,536 per cubic millimetre of blood.

It is worthy of note that there is a tendency for a high rise to be followed by a low one. This is not without exception, but the three highest rises recorded were followed by the three lowest rises.

III. CORRELATION BETWEEN THE CYCLE AND THE AMOUNT OF FEVER

From the chart one can observe that almost invariably the temperature tends to be higher during the height of the cycle, and that between the rises the temperature keeps lower.

In the table average daily temperatures are recorded. These figures were obtained by taking the average of the four-hourly temperatures observed during the twenty-four hours of each day. We think that this figure gives a fair representation of the amount of fever for the day, and in this table it may be noted that the average daily temperature rises coincidently with the increase of trypanosomes and falls with their fall.

IV. OTHER CLINICAL OBSERVATIONS AND CORRELATIONS WITH THE CYCLE

The pulse rate ranged all through this case from 90 to 120 per minute. During the height of the cycle it tended to increase coincidently with the increased amount of fever. The respiration rate ranged from 20 to 28 per minute, tending also to increase with the rise of the trypanosomes.

With regard to the bowels there is little to note. Their action was easily regulated with small doses of cascara. It was very noticeable that the patient was more drowsy, and tended to have headaches, during the commencement of the cyclical rise, but these symptoms abated at the extreme height and during the subsequent fall of trypanosomes.

The lymphatic glands in the neck, axilla, groin, and poplitcal space were more or less always swollen, but this condition was greatly aggravated at intervals, accompanied with marked tenderness, though only sometimes was this coincident with a trypanosome increase.

The urine throughout remained clear, usually slightly acid and without deposits. The specific gravity ranged from 1010 to 1026.

On March 15 he had a diffuse ill-defined erythematous rash over his left leg, occurring just before the height of a trypanosome increase.

V. CORRELATION BETWEEN THE TRYPANOSOME CYCLE AND THE BEHAVIOUR OF THE LEUCOCYTES

Previous to April 23 we made several leucocyte counts with the Thoma-Zeiss apparatus, but were unable to find anything definite. It was only when we made counts daily at the same hour that we were able to find a definite leucocyte change corresponding to the parasitic cycle.

We counted the leucocytes by means of the thick film as referred to in our paper on malaria, and were astonished at the remarkable variations. We found no such variations by our method in normal persons. Simultaneous examination of thin blood films also showed that a remarkable leucocytic variation did take place, corresponding to each rise of trypanosomes.

Daily differential counts were also made in thin films stained with Giemsa, in which we distinguished only between mononuclears and the so-called polymorphonuclears. In these we never counted less than from 300 to 500 leucocytes to ensure greater accuracy. We thus estimated daily the total leucocytes and the total numbers of polymorphs and mononuclears per cubic millimetre of blood, as shown in the chart and table.

From April 23 till May 20 it is clearly seen that coincident with each rise in the number of trypanosomes there is a marked increase in the total leucocytes, and this increase is due more to mononuclears than to polymorphonuclears.

When the trypanosomes begin to increase in number the leucocytes increase also, more especially the mononuclears. When the trypanosomes have reached their height there may be a fall in the number of leucocytes, but this is followed by a still higher rise during the fall of the trypanosomes. The leucocytes would seem to reach their highest numbers on about the third day after the height of the parasitic cycle. They then decrease rapidly for about three days to normal or much lower. When this occurs the trypanosomes again commence to multiply, and the leucocytes again increase also, so that we have both a parasitic and a corresponding leucocytic cycle.

We did not attempt to differentiate between large mononuclears and lymphocytes, but it seemed to us that both took part in the total mononuclear increase. We further observed that during and

immediately after the parasitic fall the large mononuclears were filled with vacuoles and a reddish *débris* (stained Giemsa). Many of these vacuolated mononuclear cells were of great size (30 to 40 microns in diameter). We are inclined to think that this vacuolation with reddish stained *débris* may be an indication of the ingestion of trypanosomes, although we have never observed any definite trypanosome structure within them.

F. W. Andrewes, in the Croonian Lectures,* points out that an intravenous injection of bacteria causes a temporary diminution in leucocytes, followed by a marked increase, this fall and rise being chiefly due to the so-called polymorphonuclears, the mononuclears taking little part in the phenomenon. In this disease, as in malaria, it would appear that there is a diminution followed by an increase, chiefly of the mononuclears. This tends to suggest that the polymorphonuclear leucocytes react chiefly to bacterial infections, while the mononuclears would seem to react chiefly to protozoal blood infections (D. T).

It is interesting to note that on May 20 there was a very marked mononuclear increase, coincident with a fall of trypanosomes, and accompanied by a remarkable improvement in the clinical condition of the patient; and again on June 24 the mononuclear excess changed into a very marked polymorph excess. This was coincident with the onset of pleurisy and pneumonia, from which the patient died.

In connection with the leucocyte cycle, the highest numbers recorded were 50,000 per cubic millimetre of blood, whereas the lowest was 2,800 per cubic millimetre of blood. In the latter part of the chart the leucocytic graph is altered by the injection of leucocytic extract, and by the development of an abscess and pneumonia.

The following quotation from the 'Sleeping Sickness Bulletin,' 1908, No. I, p. 5, is of interest:—'Dr. W. Thomas found that 'after an injection of atoxyl a change in the parasites became 'noticeable between the fourth and the fifth hour. They became 'sluggish and much altered in appearance, and at the same time 'there was a noticeable increase in the leucocytes. About the seventh 'hour there was a great diminution in the number of trypanosomes,

* Lancet, July, 1910

'and a coincident increase in the leucocytes, notably the phagocytes. 'At the eighteenth hour parasites were absent and could not be 'found, even after blood centrifugalisation. Instances of 'phagocytosis were observed on three occasions.'

Thomas and Breinl tried the effect of hyperleucocytic agents without good effects, but remark: 'It is quite evident that the 'leucocytes play a rôle in the decrease of parasites.'

We think that it is possible that on some occasions, at least, Thomas may have happened to give atoxyl at the time when the leucocytes were increasing and the parasites were diminishing, as we have shown to occur naturally.

VI. THE HAEMOGLOBIN AND RED CELLS

During the course of the disease, from February till the patient's death in June, the haemoglobin percentage fell more or less steadily from 85 to 70 per cent. We could not detect any variation in the amount of haemoglobin corresponding to the parasitic cycle.

The number of red cells was deficient (3,800,000 in March; 2,800,000 in June). Their numbers were not estimated frequently.

VII. THE EFFECTS OF TREATMENT BY VARIOUS DRUGS

The crucial test of a curative treatment in this disease would naturally be the effect of the treatment on the numbers of parasites, especially in our case, where the numbers were estimated daily.

In testing the value of various therapeutic agents, we have therefore taken the graph of the number of parasites as the indicator of the efficacy of that agent. We would like also to point out that in estimating the effect of treatment by this method, the blood must be examined every day, otherwise the effects recorded might be erroneous. If a drug be given at the height of a parasitic rise, and the blood examined next day or a few days later, the number of parasites would naturally be much less; to conclude from this that the drug has caused this diminution might be quite erroneous. It is obvious that no conclusion should be drawn until the numbers have been estimated daily for several weeks.

We think it may be possible that a drug such as atoxyl may by chance have been given, sometimes, just as the parasites were

naturally about to fall, and the rapid diminution attributed to the drug.

It has been stated that atoxyl did not always cause a disappearance of the parasites, but that sometimes instead they even increased in numbers; and further, that it seemed to have a more marked trypanocidal action when the parasites were very numerous.

These statements can be understood in the light of the natural cycle.

Again, if the trypanosomes increase in number immediately after the administration of a drug, one cannot at once conclude that this drug is of no value as a trypanocide.

The Effect of Atoxyl

From February 16 till April 5 atoxyl was not given, on account of its injurious effect on the patient's eyes. On April 5 we, however, injected a dose of four grains at the commencement of a natural rise. The blood was examined every two hours afterwards and showed that the trypanosomes were increasing in numbers. Twenty-four hours later there was a further marked increase. Next day again, as we would have expected to occur naturally, there was a decided diminution in their number.

On May 2 atoxyl was again given, though it was found that every dose aggravated the eye condition. From May 2 till June 20, thirty-two grains of atoxyl in all were administered at intervals, as noted on the chart. On comparing the graph where no atoxyl was given, and where it was administered, no very appreciable difference in the number of trypanosomes can be detected. We cannot, however, conclude that the atoxyl has no trypanocidal effect, as had it not been given the trypanosomes may possibly have been much more numerous during the latter than in the earlier part of the chart; and further, this particular strain of trypanosomes seems to have been very virulent.

Our doses of atoxyl in any case were rather small. It would appear to us, however, that atoxyl cannot be considered a specific in human trypanosomiasis, as quinine is in malaria. In our cases of malaria quinine never failed to diminish, markedly, the number of asexual parasites. Moreover, atoxyl compared with quinine is a dangerous drug.

Quinine and Methylene Blue Treatment

Quinine 30 grains daily, combined with methylene blue, 12 grains daily, was administered by the mouth from February 21 till March 9, and again at other intervals as noted on the chart. No marked trypanocidal effect can be noticed.

During treatment with these drugs, however, as also with atoxyl, the patient's face, which had before been puffy and oedematous, especially about the eyelids, became more firm and clear cut. The eyelids became again oedematous soon after these drugs were withheld. It would seem, therefore, that quinine and methylene blue, as well as atoxyl, had some beneficial effect clinically. The following drugs were also given without apparent results:—

(*a*) *Trypsin and Amylopsin Injections.*—20 min. of each daily.

(*b*) *Succinamide of Mercury Injection.*—One-fifth grain almost daily.

(*c*) *Izal Oil.*—8 min. daily, by mouth.

(*d*) *Trypan Red.*—By the mouth in doses of 0·5 grain, 1 grain, and 1 grain respectively on three successive days. No albuminuria resulted, but the drug was stopped on account of the development of severe vomiting.

(*e*) *Potassium Iodide.*—30 grains daily, by mouth.

We beg to apologise for complicating the case with so many treatments, but owing to the unsuitability of atoxyl in the patient other treatment was a clinical necessity.

VIII. EFFECT OF TRYPANOSOME 'VACCINES

On April 19 we commenced to give our patient subcutaneous injections of so-called vaccine. This vaccine was obtained from the blood of a rat inoculated from our patient. The rat was killed when the parasites were extremely numerous (500,000 per cubic millimetre of blood). The blood was then drawn from its heart aseptically, and mixed with an equal volume of normal saline. The red cells were allowed to settle, and the supernatant fluid pipetted off. This latter contained most of the trypanosomes and the number per cubic millimetre was estimated by the thick film method. It was then sterilised by heating to 55° C. for half-an-hour, and by adding trikresol so that it contained 0·2 per cent.

The later vaccines which we commenced to use from May 17 were simply the blood of rats, taken when the trypanosomes were very numerous. They consisted of dead trypanosomes, red cells, leucocytes, and serum. The injection of these vaccines produced no local reaction, even in doses of 100,000,000 trypanosomes; nor were we able to detect any definite temperature reaction.

We are inclined to think that the chief result of these injections of dead trypanosomes was a stimulation of the reproductive powers of the living trypanosomes. This point, however, requires further elucidation. We found that after an injection of our vaccine, the next trypanosome rise usually occurred before it was naturally due.

On April 9, 9,000,000 dead trypanosomes were injected; the next trypanosome rise reached its height on the seventh day. 20,000,000 were again injected on April 13, and the following rise was completed on the sixth day. 40,000,000 were then injected and the next rise was completed on the fifth day.

The patient's trypanosomes, also, which were rising to successively smaller heights, continued to diminish further in number, after two more injections of 10,000,000 dead trypanosomes on April 23 and 25. On April 28, however, an injection of 10,000,000 was given when the trypanosomes were increasing, and that rise was the highest recorded. This treatment was then stopped for some time.

It seems that the effect of these so-called vaccines, if injected immediately after the natural fall of the parasites, is a reproductive stimulation of the parasites, causing the next rise to occur sooner than was natural; and this premature rise tends to be less high than it probably should have been in the natural course of events. If, however, the vaccine be injected during the natural rise of the trypanosomes, it stimulates their reproduction, causing a very high and rapid rise, and the subsequent fall is also very sudden.

We confirmed these surmises later. On May 17 an injection of 30,000,000 dead trypanosomes was given, with the result that the following rise was completed on the sixth day.

Another injection of 50,000,000 on May 30 was followed by a rise at the normal time. A large dose of 100,000,000, injected on June 4, however, scarcely allowed the parasites any time to diminish, so that they completed their next rise on the fifth day, followed by

a sudden fall. Thus it would seem that the normal period of seven days between the rises of parasites may be shortened to six, five, and even four days, by an injection of dead trypanosomes.

Doses of vaccine up to 100,000,000 seemed to cause no harm. On the contrary, during the period from the 7th to the 28th of April, when small doses were given, the temperature was more uniform, and more near to normal than it had ever been before or after.

Again, from the 14th to the 19th of May, the patient was extremely ill, almost comatose, with a high temperature, and like to die. On May 17 he had vaccine (30,000,000), followed on May 19 by an injection of 10 c.c. of leucocytic extract. The improvement on May 20 was remarkable, both mentally and physically. Of course, at that time he had atoxyl and nuclein to increase the leucocytes, hence it is almost impossible to tell which was the potent factor. Against atoxyl having caused this good result, we may point out that it had no such effect either before or since.

As already stated, this marked improvement was coincident with a large increase of mononuclear leucocytes. We tried again to repeat this success by injecting vaccine (100,000,000 trypanosomes) on June 4, three days before the height of the parasitic rise, and leucocytic extract at the height of the rise, but the result was not so good. (*Vide* paper by Ross and J. G. Thomson.†)

IX. THE EFFECT OF SUBCUTANEOUS INJECTIONS OF LEUCOCYTIC EXTRACT

Thinking that the fall in the number of leucocytes might be a factor in the rise of trypanosomes, we determined to keep up the numbers of leucocytes when they were falling. Consequently, we tried an injection of nuclein (25 min.), but this did not seem to cause much increase of leucocytes. Yeast, 15 grains daily (by mouth), was also tried. It seemed to cause some increase, but not marked.

At this time Dr. Moore Alexander, pathologist to the hospital, suggested that we should try leucocytic extract. He thought that this extract might contain certain substances which would be deleterious to the trypanosomes, or which would neutralise their

† Proc. Roy. Soc., Vol. LXXXIII, 1911, p. 227.

endotoxins. He was good enough to prepare it for us by injecting Mellin's food into the pleural cavities of rabbits. The rabbits were then killed, and the accumulation of leucocytes taken from the pleural cavity, and extracted with sterile distilled water.

To our surprise, the effect of an injection of this leucocytic extract was to produce a very great increase of leucocytes in the blood on the day following the injection. This was almost the invariable result. In our opinion, it seems to be a far more powerful promoter of leucocyte increase than yeast or nuclein.

We tried to utilise this discovery in two ways:—

(i) To help the natural leucocyte increase by injecting the extract just at the height of the trypanosome rise. We did this on May 19. Next day, as we have mentioned, the patient showed a remarkable improvement. We were unable, however, to get such a happy result again.

(ii) By injecting the extract a day or two before the rise of parasites, we thought we might prevent that rise by causing an early increase in the leucocytes.

On May 31 10 c.c. of the extract were injected, producing next day an increase of leucocytes up to 20,000 per cubic millimetre of blood, but they diminished next day to 2,800 per cubic millimetre, and the increase of trypanosomes occurred all the same.

Later, just before the patient's death, we tried the effect of 1 c.c. doses of the extract daily. This seemed to keep the leucocyte count high, but unfortunately the results were complicated by the patient developing an abscess at this time, and later by the development of pleuro-pneumonia.

Further investigation with this substance, and with vaccine, would require to be made before coming to any definite conclusions. Such an investigation we think would lead to further knowledge regarding the natural balance of immunity between the body and the parasites.

It appears to be a law (at least it seems to hold good, we think, with leucocytes and trypanosomes) that the extract of dead animal cells stimulates the corresponding live cells to increase in numbers. This would appear to support the hypothesis put forward by Dr. H. C. Ross, that the extracts of dead tissues promote the proliferation of living cells.*

* Brit. Med. Journ., June 11, 1910.

X. CONCLUSIONS REGARDING THE NATURE AND CAUSE OF THE TRYPANOSOME CYCLE

The true explanation of this phenomenon must be of extreme importance, not only as regards the treatment of this disease, but also, we think, with regard to the problems of immunity in general.

Before stating our views we cannot do better than quote the following extract from the 'Sleeping Sickness Bulletin,' 1909, No. 12, p. 485:—

'*Des Causes des Crises Trypanolytic et des Rechûtes qui les suivent.*
'A MASSAGLIA. "Comptes Rendus de l'Academie des 'Sciences," Octobre, 1907.

'In some species of animals there are no crises. The 'trypanosomes increase in a progressive regular manner. In others 'the trypanosomes, after an increase, suddenly diminish to such an extent sometimes that they cannot be found in the blood. They 'soon reappear. Massaglia endeavoured to find out the cause of 'the crisis and the subsequent relapses. . . . *Conclusions:* 'Trypanolytic crises are due to the formation of anti-bodies in the 'blood. A few parasites escape destruction, because they become 'used or habituated to the action of these anti-bodies. These are 'the parasites which cause the relapses. Since the trypanosomes 'become more and more used to the anti-bodies the subsequent 'crises become less marked. . . . Thiroux's suggestion of a 'balance between anti-bodies and parasites is an interesting one 'and has some facts in its support. It would serve to explain the 'cause of real or apparent cures mentioned in Bulletin No. 5, 'pp. 193 and 195.'

Here it is clear that Massaglia has observed and has tried to explain the occurrence of these natural rises and falls of trypanosomes. Our opinions on the subject are as follows:—

(i) *The cycle is not due to an unconditional cyclical development of the parasites as is the case in malarial fever.*—If it were so then one would expect the cycle to be more regular, and one would not expect its time to be altered by vaccine injections. The time between the rises varies not only in the same individual, but in different animals. This would appear to suggest that the cycle is not due to a definite parasitic development as in malaria, but is

merely a question of a struggle between the defensive powers of the infected body and the aggressive powers of the trypanosomes.

The more susceptible the animal the shorter is the period between the rises as seen in the case of rats, in which the cycle is almost lost. Massaglia evidently failed to observe the slight remissions in the rat.

The greater the resistance of the infected animal the longer is the cyclical period, as in guinea-pig and man.

By an 'unconditional cycle' we mean one of which the *period* is not affected by the resistance of the host or by therapeutic agents—as, for instance, that of *Plasmodium*. By a 'conditional cycle' we mean one which is so affected—as in the present case.

(ii) *The increase of parasites is due to their sudden and active multiplication.*—The presence of numerous dividing forms during the cyclical rise would seem to support this statement.

(iii) *The multiplication of trypanosomes is extremely rapid.*—As before stated they seem capable of dividing in man three to four times in twenty-four hours.

In rats the rate of multiplication would seem much greater, as many as ten divisions or generations may occur in twenty-four hours.

(iv) *The rate of multiplication depends on the suitability of the infected blood to the parasite.*—The blood of rats seems more suitable to the parasite than the blood of man and guinea-pigs.

Also this suitability would appear to vary from time to time in the same animal. In our case there occurred high and low rises in the numbers of trypanosomes. Moreover the three highest rises recorded in the chart were followed by the three lowest rises recorded. This is a very significant fact. It would appear to indicate that a high rise sets up some reactionary condition. This reaction, if great, not only causes the decrease of trypanosomes but extends its influence so far as to reduce the next rise.

(v) *The fall in number of parasites is not due to the toxins they develop.*—In man the numbers may reach only 200 per cubic millimetre of blood and then they diminish, whereas in rats they may reach over 200,000 per cubic millimetre of blood and yet continue to rise, and the toxins in the latter case must be much more abundant than in the former.

It would seem, therefore, that the explanation may be reduced to some of the following causes, all of which may come into play.

(i) *The increase of trypanosomes is due to their active multiplication*, the rate of multiplication depending on the following conditions:—

(*a*) The liberation of a reproductive stimulant from the dead trypanosomes of the previous fall.

(*b*) The small number of leucocytes, especially mononuclears.

(*c*) The habituation of the trypanosomes to their anti-bodies.

(*d*) The absence or the diminution of anti-bodies to the trypanosomes.

(ii) *The decrease of trypanosomes is due to their rapid death and to a cessation of multiplication*, probably depending on the following conditions:—

(*a*) The presence of anti-bodies in the serum.

(*b*) The large increase of leucocytes, especially mononuclears.

(iii) *The trypanosomes remaining between the rises are resistant forms*, and tend to become spherical, especially in the internal organs. (*Vide* H. B. Fantham.*)

These resistant spherical forms also occur after atoxyl treatment (Moore and Breinl).

N.B.—The reference to the virulence of this trypanosome and to the cycle in rats and guinea-pigs is taken from the paper on enumerative studies in rats, guinea-pigs, and rabbits by H. B. Fantham and J. G. Thomson.†

The virulence of this strain was also noticed by L. E. W. Bevan and Malcolm Macgregor ('Journal of Comparative Pathology and Therapeutics,' June, 1910), who inoculated animals from our patient before he left N.E. Rhodesia.

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* Ann. Trop. Med. and Parasit., Vol. IV, pp. 465-485; also Proc. Roy. Soc., B, Vol. LXXXIII, pp. 212-227.

† Ann. Trop. Med. and Parasit., Vol. IV, pp. 417-463; also Proc. Roy. Soc., B, Vol. LXXXIII, pp. 206-211.

TABLE OF DAILY DETAILS

W.A.—*Sleeping Sickness.* Infected September, 1909; admitted December, 1909; age 26; male; weight 154 lbs.; died June 29, 1910

NOTE.—The parasites are given per c.mm. The temperatures are recorded in the Haematothermic Fahrenheit scale, which is the Fahrenheit scale minus 95·0 multiplied by 10. The leucocytes are given in 100's per c.mm.; and the haemoglobin in Tallqvist's scale.

	February													March		
Date	16	17	18	19	20	21	22	23	24	25	26	27	28	1	2	3
Number of Tryps. per c.mm....	—	166	195	140	296	687	800	500	254	130	70	—	124	570	606	70
Maximum temp., H.F.	60	74	60	40	58	56	54	50	30	40	56	52	56	42	54	36
Average daily temp., H.F. ...	49	57	46	35	39	44	39	31	29	27	35	28	35	37	35	29
Total Leucocytes, in 100's ...	56	44	39	50	53	—	—	—	62	—	—	—	99	—	119	—
Polymorphs, in 100's	34	26	26	—	—	—	—	—	36	—	—	—	53	—	66	—
Mononuclears, in 100's	22	18	13	—	—	—	—	—	26	—	—	—	46	—	53	—
Mononuclears. per cent.	39	44	36	—	—	—	—	—	42	—	—	—	47	—	45	—
Hb. per cent. (Tallqvist)	R.B.C., 3,800,000	—	—	—	—	—	—	—	—	—	—	—	—	—	R.B.C., 3,800,000	—
Treatment	Atoxyl, 2 gr.	—	—	$HgCl_2$, $\frac{1}{5}$ gr.	—	Quinine, 30 gr. Meth. blue, 50 c.c.	*et seq.*		—	—	—	—	—	—	—	—

	March															
Date	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Number of Tryps. per c.mm....	26 ?	12	40	204	1256	1400	472	152	32	76	160	220	320	68	180	132
Maximum temp., H.F.	34	38	36	50	46	34	38	38	36	34	44	46	38	42	40	36
Average daily temp., H.F. ...	24	24	25	33	33	28	19	27	25	22	32	33	30	29	32	27
Total Leucocytes, in 100's ...	—	—	—	—	—	—	—	—	156	—	—	—	31	47	—	73
Polymorphs, in 100's	—	—	—	—	81	—	—	—	113	—	—	—	17	24	—	43
Mononuclears, in 100's	—	—	—	—	35	—	—	—	43	—	—	—	14	23	—	30
Mononuclears, per cent.	—	—	—	—	—	—	—	—	34	—	—	—	46	49	—	41
Hb. per cent. (Tallqvist)	—	—	85	85	80	—	—	—	80	—	85	85	80	75	80	82
Treatment	—	—	—	—	—	—	—	—	Quinine stopped	—	—	—	—	Tryp. red, 5 gr. $HgCl_2$, $\frac{1}{3}$ gr.	Tryp. red, 15 gr.	Tryp. red, 15 gr.

	March												April			
Date	20	21	22	23	24	25	26	27	28	29	30	31	1	2	3	4
Number of Tryps. per c.mm....	32	24	100	620	1136	322	68	72	32	188	748	1016	144	52	52	40
Maximum temp., H.F.	32	32	40	52	44	38	32	38	28	46	50	42	60	48	32	32
Average daily temp., H.F. ...	27	21	31	40	37	29	26	24	23	31	37	30	31	35	19	25
Total Leucocytes, in 100's ...	—	62	—	45	73	99	83	56	80	47	41	100	47	81	53	76
Polymorphs, in 100's	—	38	—	29	28	57	34	23	47	25	16	?	21	36	21	37
Mononuclears, in 100's	—	24	—	16	45	42	49	33	33	22	25	?	26	45	32	39
Mononuclears, per cent.	—	40	—	35	62	43	60	59	41	46	62	?	55	56	60	51
Hb. per cent. (Tallqvist)	85	85	85	82	85	85	85	—	85	85	85	80	80	80	80	80
Treatment	—	—	—	Trypsin, amylopsin	*et seq.*	—	Vaccine, 1,000,000	—	—	—	Quinine, 20 gr.	*et seq.* Izal oil, 8 min.	Izal oil, 8 min.	Izal oil, 8 min.	—	—

	April													
Date	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Number of Tryps. per c.mm....	212	680	1164	340	116	172	416	556	650	192	96	172	116	392
Maximum temp., H.F.	52	46	40	34	32	30	40	40	40	30	30	38	40	40
Average daily temp., H.F. ...	37	28	27	23	19	21	33	26	25	21	25	25	24	31
Total Leucocytes, in 100's ...	61	56	89	59	84	53	40	70	62	81	52	?	—	—
Polymorphs, in 100's	33	34	44	35	59	22	19	45	—	41	32	?	—	—
Mononuclears, in 100's	28	22	44	24	25	31	21	25	—	40	20	?	—	—
Mononuclears, per cent.	46	40	50	41	30	58	53	36	—	49	39	47	49	48
Hb. per cent. (Tallqvist)	85	85	85	80	80	80	80	80	85	85	85	85	—	85
Treatment	Atoxyl, 4 gr.	—	—	—	Vaccine, 9,000,000	—	—	—	Succ. Hg, $\frac{1}{5}$ gr.	Atoxyl, 2 gr.	Atoxyl, 2 gr. Vaccine, 2,000,000	—	—	Succ. Hg. $\frac{1}{5}$ gr.

	April											
Date	19	20	21	22	23	24	25	26	27	28	29	30
Number of Tryps. per c.mm....	336	90	72	244	156	164	88	48	140	200	1536	120
Maximum temp., H.F.	36	32	52	44	34	32	38	38	32	32	52	42
Average daily temp., H.F. ...	23	22	31	34	27	24	27	27	24	25	32	30
Total Leucocytes, in 100's ...	100	—	—	—	100	130	100	72	120	240	108	140
Polymorphs, in 100's	52	—	—	—	60	78	70	—	64	144	52	66
Mononuclears, in 100's	48	—	—	—	40	52	30	—	56	96	56	74
Mononuclears, per cent.	48	36	31	52	40	40	30	—	46	40	52	53
Hb. per cent. (Tallqvist)	85	—	80	85	80	80	80	80	85	80	85	—
Treatment	—	Quinine stopped. Vaccine, 40,000,000	—	—	Vaccine, 10,000,000	—	Vaccine, 10,000,000	—	—	Vaccine, 10,000,000	—	Succ. Hg. $\frac{1}{5}$ gr.

	May										
Date	1	2	3	4	5	6	7	8	9	10	11
Number of Tryps. per c.mm....	64	64	68	76	292	8	40	108	56	64	8
Maximum temp., H.F.	38	34	40	48	44	46	46	34	54	44	46
Average daily temp., H.F. ...	24	28	30	34	32	24	27	26	35	25	31
Total Leucocytes, in 100's ...	500	110	53	73	210	174	230	350	150	75	62
Polymorphs, in 100's	265	70	33	40	101	87	138	168	63	25	25
Mononuclears, in 100's	235	40	20	33	109	87	92	182	87	50	37
Mononuclears, per cent.	47	36	38	45	52	50	40	52	58	66	59
Hb. per cent. (Tallqvist)	85	80	80	80	80	80	80	80	80	80	80
Treatment	—	Atoxyl, 2 gr.	Succ. Hg, $\frac{1}{5}$ gr.	—	Succ. Hg, $\frac{1}{5}$ gr. Atoxyl, 2 gr.	—	Succ. Hg, $\frac{1}{5}$ gr.	—	Succ. Hg, $\frac{1}{5}$ gr. Quin. et Meth. blue.	—	—

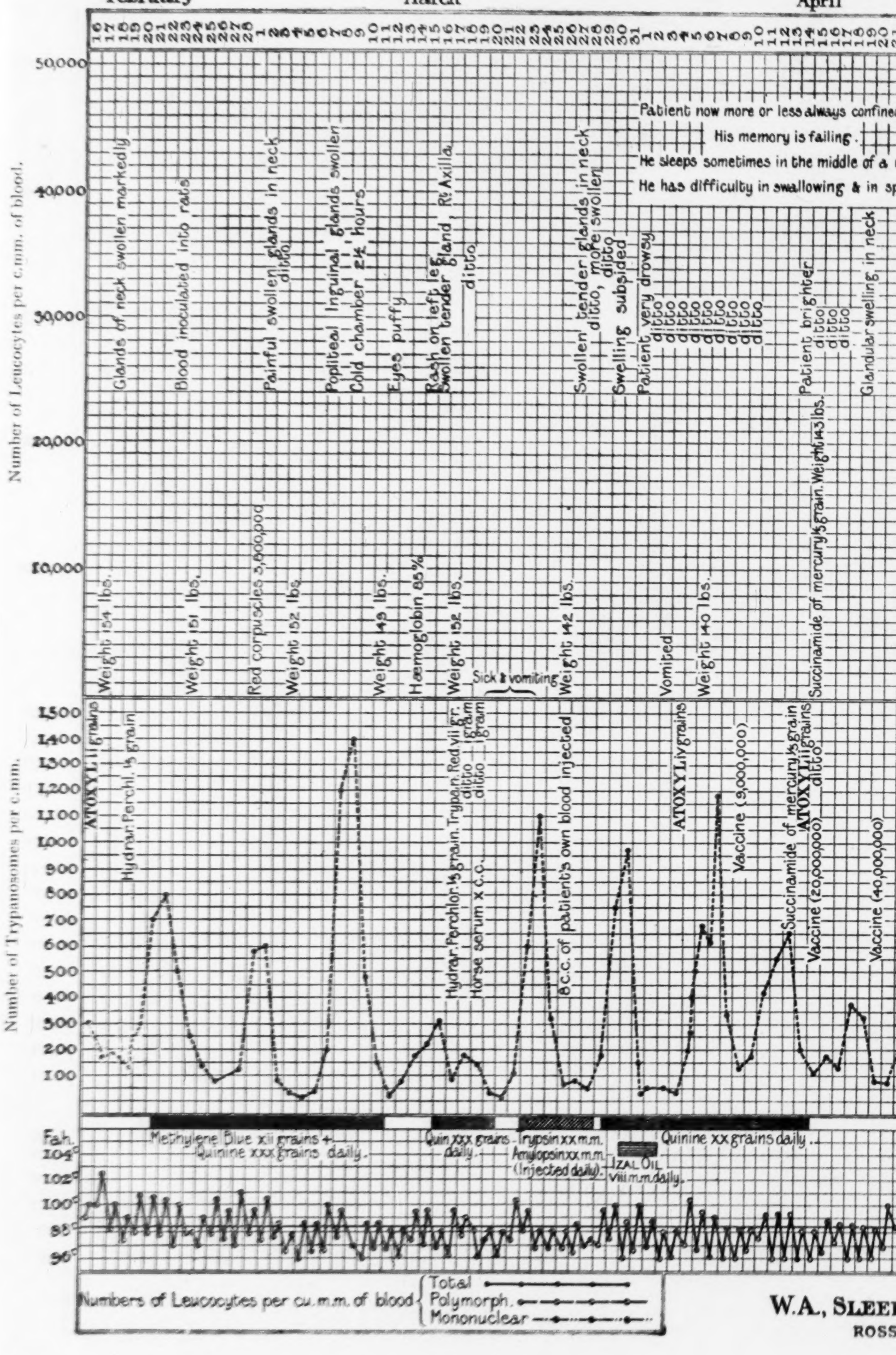
	May									
Date	12	13	14	15	16	17	18	19	20	21
Number of Tryps. per c.mm....	76	100	700	8	4	52	176	900	208	104
Maximum temp., H.F.	56	56	68	48	48	56	70	78	64	42
Average daily temp., H.F. ...	38	36	48	30	34	41	47	64	45	35
Total Leucocytes, in 100's ...	45	63	37	75	157	36	72	68	500	100
Polymorphs, in 100's	16	—	33	21	71	18	33	29	160	38
Mononuclears, in 100's	29	—	33	54	86	18	39	39	340	62
Mononuclears, per cent.	64	—	50	72	55	49	54	57	68	62
Hb. per cent. (Tallqvist)	80	75	80	80	80	80	80	80	80	85
Treatment	Succ. Hg, $\frac{1}{5}$ gr. Atoxyl, 2 gr.	—	Succ. Hg. $\frac{1}{5}$ gr.	—	—	Vaccine, 30,000,000 Nuclein, 25 min.	Atoxyl, 2 gr.	Atoxyl, 2 gr. Leuc. ext., 10 c.c.	—	—

	May										June
Date	22	23	24	25	26	27	28	29	30	31	1
Number of Tryps. per c.mm....	104	112	68	120	150	552	416	100	48	72	128
Maximum temp., H.F.	42	42	44	36	30	50	42	34	36	36	44
Average daily temp., H.F. ...	36	28	31	23	24	38	27	27	25	27	34
Total Leucocytes, in 100's ...	96	140	53	170	175	54	80	57	43	30	200
Polymorphs, in 100's	39	71	30	114	117	21	34	26	12	13	116
Mononuclears, in 100's	57	69	23	56	58	33	46	31	31	17	84
Mononuclears, per cent.	59	49	44	33	33	62	58	55	72	56	42
Hb. per cent. (Tallqvist)	85	—	85	85	85	85	85	85	80	80	80
Treatment	—	—	Atoxyl, 2 gr. Leuc. ext., 10 c.c.	Atoxyl, 2 gr.	—	—	—	—	Vaccine, 50,000,000	Leuc. ext., 10 c.c.	Atoxyl, 3 gr.

	June										
Date	2	3	4	5	6	7	8	9	10	11	12
Number of Tryps. per c.mm....	240	352	248	112	468	820	20	12	24	208	1164
Maximum temp., H.F.	44	56	54	30	38	56	40	40	46	56	58
Average daily temp., H.F. ...	34	33	36	21	24	39	27	26	30	41	48
Total Leucocytes, in 100's ...	28	40	300	270	110	45	200	80	240	108	160
Polymorphs, in 100's	14	23	114	119	45	27	72	33	130	49	74
Mononuclears, in 100's	14	17	186	151	65	18	128	47	110	59	86
Mononuclears, per cent.	50	43	62	56	59	40	64	59	46	55	54
Hb. per cent. (Tallqvist)	80	80	80	80	80	80	80	80	80	80	80
Treatment	Atoxyl, 3 gr.	—	Vaccine, 100,000,000	—	Leuc. ext., 4 c.c.	Leuc. ext., 4 c.c.	—	Vaccine, 100,000,000	—	—	—

	June							
Date	13	14	15	16	17	18	19	20
Number of Tryps. per c.mm....	16	32	8	32	16	100	1452	680
Maximum temp., H.F.	60	58	50	38	38	50	50	60
Average daily temp., H.F. ...	48	41	40	27	28	37	44	45
Total Leucocytes, in 100's ...	310	200	280	100	250	81	250	37
Polymorphs, in 100's	124	92	101	40	125	39	90	29
Mononuclears, in 100's	186	108	179	60	125	42	160	16
Mononuclears, per cent.	60	54	64	60	50	52	64	43
Hb. per cent. (Tallqvist)	80	80	—	—	80	—	80	80
Treatment	Atoxyl, 4 gr.	Atoxyl, 4 gr.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Atoxyl, 4 gr.	Atoxyl, 4 gr.

	June								
Date	21	22	23	24	25	26	27	28	29
Number of Tryps. per c.mm....	56	72	40	24	300	260	40	40	30
Maximum temp., H.F.	42	42	34	40	66	88	78	74	70
Average daily temp., H.F. ...	35	34	30	27	42	75	70	69	70
Total Leucoctes in 100's	32	120	60	210	195	84	500	126	—
Polymorphs, in 100's	13	61	28	147	78	66	380	105	—
Mononuclears, in 100's	19	59	32	63	117	18	120	21	—
Mononuclears, per cent.	60	49	54	35	60	21	24	17	—
Hb. per cent. (Tallqvist)	80	70	70	70	75	70	70	70	—
Treatment	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	Leuc. ext., 1 c.c.	—	—	—	—


February
March
April
Number of Leucocytes per c.mm. of blood.
50,000
40,000
30,000
20,000
10,000
Weight 154 lbs.
Glands of neck swollen markedly
Weight 151 lbs.
Blood inoculated into rats
Red corpuscles 3,600,000
Painful swollen glands in neck
Weight 152 lbs.
ditto
Weight 149 lbs.
Popliteal Inguinal glands swollen
Cold chamber 24 hours
Hæmoglobin 85%
Eyes puffy
Weight 152 lbs.
Rash on left leg
Swollen tender gland, Rt Axilla
ditto
Weight 142 lbs.
Swollen tender glands in neck
ditto, more swollen
Swelling subsided
Patient very drowsy
ditto
Weight 140 lbs.
Vomited
Sick & vomiting
Succinamide of mercury ⅓ grain. Weight 143 lbs.
Patient brighter
Glandular swelling in neck
Patient now more or less always confine
His memory is failing
He sleeps sometimes in the middle of a
He has difficulty in swallowing & in sp
Number of Trypanosomes per c.mm.
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
ATOXYL ii grains
Hydrar. Perchl. ½ grain
Hydrar. Perchlor. ½ grain. Trypan. Red vii gr.
ditto 1 gram
ditto 1 gram
Horse serum x c.c.
8 c.c. of patient's own blood injected
ATOXYL iv grains
Vaccine (9,000,000)
Succinamide of mercury ⅓ grain
ATOXYL ii grains
Vaccine (20,000,000)
ditto
Vaccine (40,000,000)
Fah.
104°
102°
100°
98°
96°
Methylene Blue xii grains +
Quinine xxx grains daily.
Quin xxx grains daily.
Trypsin xx m.m.
Amylopsin xx m.m.
(Injected daily).
IZAL OIL
viii m.m. daily.
Quinine xx grains daily.
Numbers of Leucocytes per cu. m.m. of blood
Total
Polymorph.
Mononuclear
W.A., SLEE
ROSS

May June

confined to bed.
of a meal.
in speaking.

Patient has delusions
Sleeping heavily
Swollen glands in neck
Headaches
Sleeping heavily - Neuritis - Pains in feet
Legs swollen & œdematous
Patient incontinent passes urine & fæces involuntarily.
Sleeping heavily. Extremely ill, like to die
Patient like to die
Nuclein 3i. t.i.d.
Sudden remarkable improvement
Sleeping less heavily

Vaccine (40,000,000)
Vaccine (10,000,000)
Vaccine (10,000,000)
Vaccine (10,000,000)
Succinamide of mercury ½ grain
ATOXYL ii grains
Succinamide of mercury ½ grain
ATOXYL ii grms.
Succinamide of mercury ½ grain
Succinamide of mercury ½ grain
Succinamide of mercury ½ grain
ATOXYL ii grms.
Succinamide of mercury ½ grain
Nuclein xxv m.m. injected. Vaccine (30,000,000)
Leucocyte extract 10 c.c.
ATOXYL ii grains
ATOXYL ii grains
Leucocyte Ext. 10 c.c.
ATOXYL ii grains
ATOXYL ii grains
Vaccine (50,000,000)
Leucocyte Ext. 10 c.c.
ATOXYL iii grains
ATOXYL iii grains
Vaccine (100,000,000)
Leucocyte Ext. 6 c.c.
Leucocyte Ext. 4 c.c.
Vaccine (100,000,000)
ATOXYL iv grains
ATOXYL iv grains
Leucocyte Ext. 1 c.c.
Leucocyte Ext. 1 c.c.
Leucocyte Ext. 1 c.c.
Leucocyte Ext. 1 c.c.
ATOXYL iv grains
ATOXYL iv grains
Leucocyte Ext. 1 c.c.
Leucocyte Ext. 1 c.c.
Leucocyte Ext. 1 c.c.
Leucocyte Ext. 1 c.c.
Leucocyte Ext. 1 c.c.

H-70

Pleurisy
Pneumonia

Death

Meth. Blue xii grains +
Quin. xx grs. daily.
Yeast grs. v. t.i.d.
Potass. Iodidi. grs. x. t.i.d.
Abscess forming. – Abscess evacuated.

Numbers of Trypanosomes per cu. m.m. of blood
Temperature Fahrenheit

EEPING SICKNESS. (1910)
OSS AND THOMSON

(Copied by H. S. Leeson)

ENUMERATIVE STUDIES ON *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE* IN RATS, GUINEA-PIGS, AND RABBITS; PERIODIC VARIATIONS DISCLOSED*

BY
H. B. FANTHAM, D.Sc., B.A.,
AND
J. G. THOMSON, M.A., M.B., Ch.B.

(*Received for publication* 15 *October*, 1910)

CONTENTS

INTRODUCTION

The results recorded in this memoir were obtained chiefly from investigations on animals—rats, guinea-pigs and rabbits—sub-inoculated from a case of Rhodesian sleeping sickness recently studied by Major R. Ross and Dr. D. Thomson in the Royal Southern Hospital, Liverpool.

The investigations were undertaken at the suggestion of Major Ross, under funds allotted by the Tropical Diseases Research

* An abstract of this paper was read before the Royal Society on December 8, 1910, and published in Proc. Roy. Soc., B, Vol. LXXXIII, pp. 206-211.

Committee in the case of one of us (H.B.F.), and under the Sir Edwin Durning-Lawrence Fund in the case of the other (J.G.T.). The researches on animals were necessary in order to determine whether a regular periodic increase in the parasites in the peripheral circulation occurred, similar to that found by R. Ross and D. Thomson* (1910) in the case of Rhodesian sleeping sickness. Again, it was necessary to determine whether this numerical cyclical development had a definite significance in the life-history of the parasite, which it would be necessary to consider in the case of treatment of trypanosomiasis by drugs or other methods. Our work was done in the Liverpool School of Tropical Medicine.

The trypanosome from the patient suffering from sleeping sickness contracted in Rhodesia was found by Stephens and Fantham (1910)† to exhibit a marked morphological peculiarity in the presence of a posterior nucleus in some of the stout forms, and for this parasite the name *T. rhodesiense* was suggested by them. We have also used an old laboratory strain of *T. gambiense*.

OUTLINE OF THE METHODS USED

In all cases a definite number of trypanosomes was inoculated. The inoculation was made subcutaneously in rats, intraperitoneally in guinea-pigs and rabbits.

The peripheral blood of these animals was then examined daily, at the same hour, that is to say, at regular intervals of twenty-four hours.

The technique employed was that elaborated by R. Ross and D. Thomson (1910) in their study of the patient W.A., namely, that a known quantity of blood (one cubic millimetre divided into quarters) was taken and the parasites therein very carefully counted. In counting the thick films an Ehrlich eye-piece was used, after the films had been dehaemoglobinised, fixed with absolute alcohol and stained by the Romanowsky method.

When the parasites became very numerous it was necessary to spread the quarter cubic millimetre of blood over a larger square area than usual, and to use a very small diaphragm in the eye-piece. The parasites were then counted in three or four bands across the film, which had been previously examined in order to ascertain an

* Proc. Roy. Soc., B, Vol. LXXXII, pp. 411-415, and Vol. LXXXIII, pp. 187-205.
† Proc. Roy. Soc., B. Vol. LXXXIII, pp. 28-33.

even distribution of the trypanosomes therein—though this led to certain unavoidable errors (see R. Ross and D. Thomson on 'Enumerative Studies on Malarial Fever'*). It will be obvious that the counts entailed much labour, especially when the parasites were numerous.

We have endeavoured to estimate the number of divisions of the trypanosomes during the first twenty-four hours of their appearance in the peripheral blood of the host. (See pp. 433-4.)

ENUMERATIONS IN RATS

We now give a series of tables of enumerations in rats inoculated with (*a*) *T. rhodesiense* and (*b*) *T. gambiense*.

In the case of both strains it was found that the rats tended to fall into one of two main categories exhibiting either (1) a periodic increase and decrease in the number of parasites in the peripheral blood (see Chart 1), or (2) a more or less continuous rise in the numbers of parasites until the animal died (see Chart 2). (Cf. Moore and Breinl†.)

We therefore classify the animals inoculated with each strain into two groups:—

(*a*) *Trypanosoma rhodesiense*

I. RATS EXHIBITING PERIODIC VARIATION

RAT 1.—Piebald ♂, weight 187 grams. Dose of inoculation one million Trypanosomes. (H.B.F.)

Days	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	6	48	9,860	60,000	84,640
Temp.[1]	30	32	44	64	64	50	50

Day	8	9	10	11	12	13	14
Parasites per c.mm.	7,760	24,000	20,480	63,800	32,000	21,760	89,600
Temp.[1]	28	10	2	30	22	0	10

[1] The temperature is expressed according to the following Haematothermic scale, as in Major Ross's recent papers on 'Malaria' and 'Trypanosomiasis':—Temp. = (F − 95) × 10, where F is the temperature in degrees Fahrenheit, recorded with a clinical thermometer, per rectum.

* Proc. Roy. Soc., B, Vol. LXXXIII, p. 161, and Ann. Trop. Med. and Parasitol. (1910), Vol. IV, p. 268.

† Ann. Trop. Med. and Parasitology (1907), Vol. I. p. 448.

The graph of the number of parasites of this animal is represented in Chart 1.

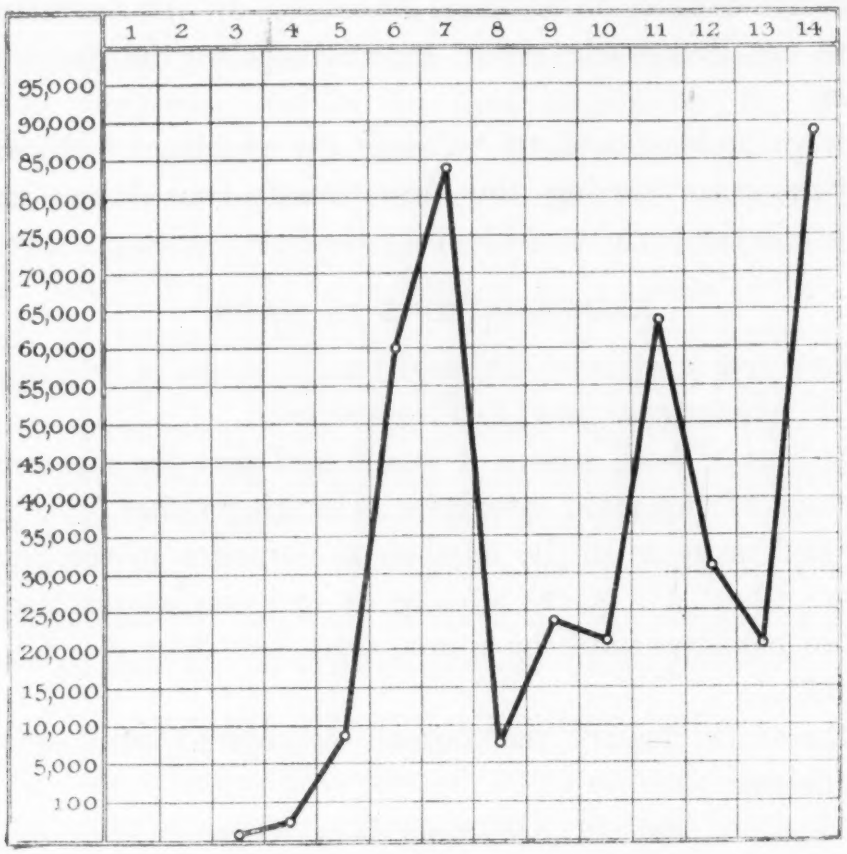


CHART 1.—Graph showing Daily Counts of the Parasites per c.mm. in the Peripheral Blood of a Rat inoculated with *T. rhodesiense*. Periodic Variation.

The numbers of the parasites are indicated along the vertical line, and the duration of infection in days along the horizontal line.

(See record of Rat 1.)

RAT 2.—White ♂, weight 108 grams. Dose 350,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	68	18,240	44,120	32,080	22,400
Temp.	32	24	20	40	56	38	22

Day	8	9	10	—	—	—	—
Parasites per c.mm.	12,860	126,000	61,840	—	—	—	—
Temp.	20	25	4	—	—	—	—

Rat 3.—White ♀, weight 225 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	12	3,480	58,240	16,000	1,840	6,400
Temp.	20	20	14	86	106	40	15

Day	8	9	—	—	—	—	—
Parasites per c.mm.	52,000	42,400	—	—	—	—	—
Temp.	15	0	—	—	—	—	—

Rat 4.—Piebald ♂, weight 220 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	8	1,960	11,520	28,800
Temp.	40	53	50	50	34	36	74

Day	8	9	10	11	12	13	—
Parasites per c.mm.	56,320	84,000	50,000	39,200	160,000	100,400	—
Temp.	50	48	38	40	47	20	—

Rat 5.—White ♀, weight 120 grams. Dose: 750,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	896	3,280	32,560	51,600
Temp.	38	36	38	50	40	52	48

Day	8	9	10	11	—	—	—
Parasites per c.mm.	64,800	22,800	16,800	6,160	—	—	—
Temp.	44	22	20	10	—	—	—

RAT 6.—Piebald ♂, weight 100 grams. Dose: one million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	32	8,400	52,728	67,760
Temp.	40	40	46	56	60	50	14

Day	8	9	—	—	—	—	—
Parasites per c.mm.	96,000	40,656	—	—	—	—	—
Temp.	30	54	—	—	—	—	—

RAT 7.—Piebald ♂, weight 189 grams. Dose: 30,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	336	6,572	23,888	80,640	40,600
Temp.	38	40	70	72	66	70	44

Day	8	9	—	—	—	—	—
Parasites per c.mm.	30,250	150,000	—	—	—	—	—
Temp.	42	5	—	—	—	—	—

RAT 8.—White ♀, weight 195 grams. Dose: 100,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	100	0	896	60,000
Temp.	40	42	40	40	60	64	74

Day	8	9	10	11	12	13	—
Parasites per c.mm.	81,000	9,200	1,960	7,840	23,040	83,200	—
Temp.	55	56	70	60	68	52	—

RAT 9.—Piebald ♂, weight 100 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	20	4,672	8,040	70,400
Temp.				30	40	50	54

Day	8	9	10	11	12	—	—
Parasites per c.mm.	32,400	3,360	29,880	88,800	113,520	—	—
Temp.	58	46	42	40	35	—	—

RAT 10.—Piebald ♂, weight 150 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	12	3,620	8,400	2,520	7,280
Temp.	36	34	42	50	42	66	48	38

Day	9	10	11	12	13	14	15
Parasites per c.mm.	12,000	6,200	7,040	11,000	80,000	18,432	120,000
Temp.	46	34	22	40	44	24	20

Note slow rise in numbers of parasites.

RAT 11.—White ♀, weight 148 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	316	1,920	8,800	11,200
Temp.						54	66

Rat 11—continued.

Day	8	9	10	11	12	13	14
Parasites per c.mm.	2,580	1,040	52	1,032	2,348	26,400	54,000
Temp.	66	52	50				

Day	15	16	17	18	—	—	—
Parasites per c.mm.	7,360	12,120	31,460	85,460	—	—	—
Temp.					—	—	—

Note the slow rise, and the long period between the crests of the first waves, due probably to the great resistance of the animal.

Rat 12.—White ♀, weight 138 grams. Dose : 600,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	16	3,620	13,740
Temp.	34	42	54	44	54	74	66

Day	8	9	10	11	12	—	—
Parasites per c.mm.	34,272	6,048	3,640	24,360	21,600	—	—
Temp.	64	60	40	63	46	—	—

This animal was pregnant, and gave birth to five young rats on the tenth day.

Rat 13.—White, weight 140 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	84	8,928

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	0	0	560	0	7,004	1,408	2,856

Rat 13—continued.

Day	15	16	—	—	—	—	—
Number of Trypanosomes per c.mm.	118,272	162,288	—	—	—	—	—

This animal was pregnant, and gave birth to eight young rats on ninth day.

Rat 14.—Piebald, weight 127 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	1,440	45,600	66,240	7,144

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	50,232	77,440	250,000	464,640	500,000	—	—

Rat 15.—Piebald, weight 104 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	4,620	45,440

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	24,472	44,352	194,128	410,000	—	—	—

Rat 16.—White, weight 58 grams. Dose of inoculation = 200,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	104	37,000	30,240
Temp.		42	44	36		44	46

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	126,000	86,640	121,600	189,000	160,800	—	—
Temp.	34	52	20	34	18	—	—

Note 'staircase' rise in the numbers of the parasites.

RAT 17.—White, weight 62 grams. Dose of inoculation = 38,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm.	—	—	—	—	1,500	500	1,380	4,028
Temp.		50	30	56	50			70

Note initial fall in numbers during first 24 hours.

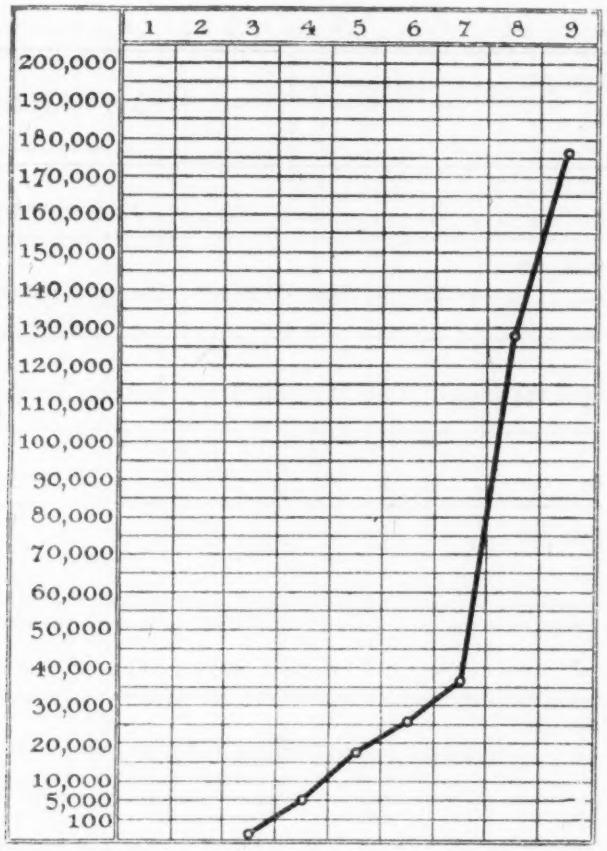


CHART 2.—Graph of Daily Counts of the Parasites in the Blood of a Rat inoculated with *T. rhodesiense*. Continuous rise. (See record of Rat 18.)

II.—RATS EXHIBITING CONTINUOUS RISE IN THE NUMBER OF PARASITES

RAT 18.—White ♂, weight 108 grams. Dose of inoculation: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	20	4,888	17,940	26,240	36,000
Temp.			34	42	50	48	30

Rat 18—continued.

Day	8	9	—	—	—	—	—
Parasites per c.mm.	129,024	175,200	—	—	—	—	—
Temp.		46	—	—	—	—	—

The graph of Rat 18 is represented in Chart 2.

Rat 19.—Piebald, weight 137 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	48	4,920	9,800	26,880
Temp.	40	40	52	62	50	62	52

Day	8	9	10	—	—	—	—
Parasites per c.mm.	31,000	51,080	160,000	—	—	—	—
Temp.	44	44	30	—	—	—	—

Rat 20.—Piebald ♂, weight 182 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	20	4,352	6,640	7,776	20,088	126,000
Temp.	36	36	34	52	48	47	10	0

Rat 21.—Piebald ♂, weight 130 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	36	3,888	42,200	58,800
Temp.	38	36	38	50	34	58	20

Day	8	9	10	11	—	—	—
Parasites per c.mm.	86,832	97,200	112,000	144,000	—	—	—
Temp.	16	50	20	0	—	—	—

RAT 22.—White ♀, weight 125 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	244	18,000	66,560	81,000	176,800
Temp.	20	22	30	26	20	6	0

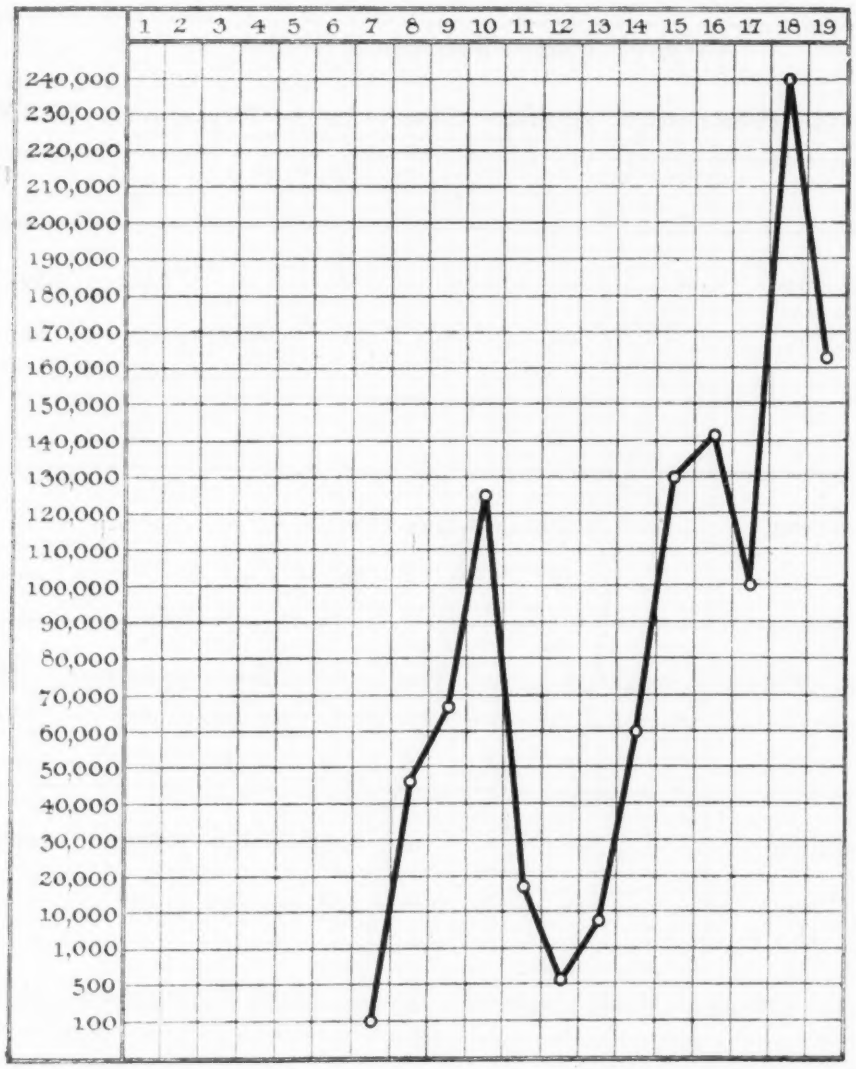


CHART 3.—Graph showing Daily Counts of the Parasites per c.mm. in the Peripheral Blood of a Rat inoculated with *T. gambiense*. Periodic Variation. (See record of Rat 23.)

(*b*) *Trypanosoma gambiense*

I.—RATS EXHIBITING PERIODIC VARIATION

RAT 23.—Piebald, weight 173 grams. Dose of inoculation: two million Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	120
Temp.	55	40	50	50	44	34	51

Rat 23—continued.

Day	8	9	10	11	12	13	14
Parasites per c.mm.	48,000	68,728	125,860	18,764	600	9,280	60,712
Temp.	45	65	50		50	66	38

Day	15	16	17	18	19	—	—
Parasites per c.mm.	130,000	142,000	100,000	240,000	164,000	—	—
Temp.	34	40	55		11	—	—

The graph of Rat 23 is represented in Chart 3.

Rat 24.—White ♂, weight 165 grams. Dose of inoculation: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	4	6,000	1,920
Temp.		30	34	34	60	64	74	52

Day	9	10	11	12	13	14	15
Parasites per c.mm.	120	200	300	360	28,800	16,800	138,240
Temp.	54		20	54	54	58	10

Rat 25.—Piebald ♂, weight 155 grams. Dose: two million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	3,080	17,080	90,000	160,000
Temp.		50	22	10	44		66

Day	8	9	10	11	12	13	—
Parasites per c.mm.	19,100	7,800	7,800	10,080	76,800	38,800	—
Temp.	20	42	40	10	0	0	—

Rat 26.—White ♀, weight 150 grams. Dose : 150,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	—	4	1,068
Temp.	48	55	46	65	60	62	58	54

Day	9	10	11	12	13	14	15
Parasites per c.mm.	4,892	768	1,680	2,860	9,688	6,384	2,436
Temp.	61	60	44	46	57	54	60

Rat 27.—White, weight 113 grams. Dose of inoculation : 60,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	120	14,310	101,740
Temp.	61	49	32	12	44		5	40

Day	9	10	11	12	13	14	15
Parasites per c.mm.	146,880	69,400	23,276	55,224	67,096	57,552	6,120
Temp.	44	36	36	35			

Rat 28.—White, weight 101 grams. Dose of inoculation : 60,000 Trypanosomes. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	256	2,808
Temp.	59	70	43	42	40		56

Day	8	9	10	11	12	13	14
Parasites per c.mm.	4,416	3,648	24,904	90,560	118,700	143,520	270,000
Temp.	44	42	34	26	24		40

Rat 28—continued.

Day	15	16	17	—	—	—	—
Parasites per c.mm.	100,000	200,600	6,048	—	—	—	—
Temp.	78	42	—	—	—	—	—

Rat 29.—Piebald ♂, weight 230 grams. Dose: one million Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	364	3,328	6,600	28,080	12,600
Temp.	36	40	56	60	47	82	56

Day	8	9	10	11	—	—	—
Parasites per c.mm.	2,808	16,380	19,360	150,000	—	—	—
Temp.	54	52	44	10	—	—	—

II.—RATS EXHIBITING CONTINUOUS RISE IN THE NUMBER OF PARASITES

Rat 30.—Piebald ♀, weight 125 grams. Dose: 500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Temp.	46	76	72		56	50	48

Day	8	9	10	11	12	13	14
Parasites per c.mm.	—	8	2,112	2,600	3,120	150,000	153,000
Temp.	62	65	42		34	58	26

Day	15	16	—	—	—	—	—
Parasites per c.mm.	181,440	540,000	—	—	—	—	—
Temp.	36	10	—	—	—	—	—

RAT 31.—Piebald ♂, weight 100 grams. Dose: 85,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	3,000	12,000	15,120
Temp.		34		30	34	32	46

Day	8	9	10	11	12	—	—
Parasites per c.mm.	24,000	101,400	200,600	378,560	216,000	—	—
Temp.	34	44	42	20	10	—	—

RAT 32.—Piebald ♀, weight 155 grams. Dose: 1,500,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	1,680	14,560	30,200	61,250	90,000
Temp.	34		34	66	52	50	44

Day	8	9	10	11	—	—	—
Parasites per c.mm.	220,500	409,600	504,000	360,000	—	—	—
Temp.	50	44	34	10	—	—	—

RAT 33.—White ♀, weight 40 grams. Dose: 100,000 Trypanosomes. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	32	8,100	48,150	88,000	126,000	180,000
Temp.			34		45		30	70

This rat was young. It is interesting to note that a count was made on the third day, five hours after the first count recorded in the table. There were then 544 parasites per c.mm., or $\frac{544}{32} = 17$ times the first count, showing that four divisions of the parasite had taken place in five hours.

From these tables it will be seen that young and light rats, such as Numbers 18 and 33, are less resistant than older and heavier rats, e.g., Numbers 1 and 23.

It will also be seen that when the initial rise is rapid and high the rat soon dies, and does not show periodic variation.

COMPARISON OF THE TWO STRAINS IN RATS

We now append a table, for purposes of easy comparison, of various points regarding the two strains in rats. The figures are compiled from the foregoing data, and relate to enumerations in seventeen rats exhibiting periodic variation, and of five rats exhibiting continuous rise in the case of *T. rhodesiense*; also of seven rats exhibiting periodic variation and of four rats showing continuous rise in the case of *T. gambiense*.

(*a*) *T. rhodesiense*

No.	Colour	Weight in grams	Incubation period in days	Duration of life in days	No. of Trypanosomes inoculated	Approximate No. of Divisions in first 24 hours	Remarks
1	Piebald	187	2	14	1 million	3	(7 to 8 divisions in next 24 hours)
2	White	108	2	10	350,000	7	
3	White	225	1	9	2 million	8	
4	Piebald	220	3	13	500,000	8	
5	White	120	3	11	750,000	2	
6	Piebald	100	3	9	1 million	8	
7	Piebald	189	2	9	30,000	4	
8	White	195	3	13	100,000	Fall	(10 divisions in next 24 hours)
9	Piebald	100	3	12	500,000	8	
10	Piebald	150	3	15	500,000	8	
11	White	148	3	18	500,000	3	
12	White	138	4	12	600,000	8	Pregnant
13	White	140	4	16	200,000	4	Pregnant
14	Piebald	127	3	12	200,000	5	
15	Piebald	104	4	11	200,000	10	
16	White	58	4	12	200,000	8	
17	White	62	4	8	38,000	Fall	
18	White	108	2	9	2 million	8	See Chart 2
19	Piebald	137	3	10	1½ million	7	
20	Piebald	182	2	8	2 million	8	
21	Piebald	130	3	11	1½ million	7	
22	White	125	2	7	1½ million	6	
				Average ...	780,000		

(β) *T. gambiense*

No.	Colour	Weight in grams	Incubation period in days	Duration of life in days	No. of Trypanosomes inoculated	Approximate No. of Divisions in first 24 hours	Remarks
23	Piebald	173	6	19	2 million	8	See Chart 3
24	White	165	5	15	500,000	10	
25	Piebald	155	3	13	2 million	3	
26	White	150	6	15	150,000	8	
27	White	113	5	15	60,000	7	
28	White	101	5	17	60,000	3	
29	Piebald	230	2	11	1 million	3	
30	Piebald	125	8	16	500,000	8	
31	Piebald	100	4	12	85,000	2	
32	Piebald	155	2	11	1,500,000	3	Not very resistant
33	White	40	2	8	100,000	8	Young
				Average ...	723,000		

From the foregoing tables it follows:—

(1) That the average incubation period* in the case of *T. rhodesiense* was 2·9 days, whereas the average incubation period in the case of *T. gambiense* was 4·4 days.

(2) The average life of rats inoculated with *T. rhodesiense* was 11·3 days, whereas the life of rats infected with *T. gambiense* was 13·8 days.

These inferences are legitimate, for the average weight of the rats inoculated with *T. rhodesiense* was 138·8 grams, whereas the average weight of those inoculated with *T. gambiense* was 137 grams. Also the numbers of trypanosomes inoculated were nearly the same in the two strains, averaging 780,000 and 723,000 respectively.

(3) On comparing the number of divisions of parasites occurring in the first twenty-four hours in both strains, we find that it was approximately the same, namely, six divisions on the average, and varied in each case from two to ten divisions, most frequently being eight.

(4) There is also a shorter period between the crests of the waves in the graphs denoting the number of trypanosomes in

* By incubation period we mean the interval (in days) from the moment of inoculation of the parasites into the host till the time the trypanosomes are first found in one cubic millimetre of the peripheral blood of host.

the Rhodesian strain (about three to four days) than in the laboratory strain of *T. gambiense* (viz., four to six days). (See Charts 1 and 3.)

(5) The difference in morphology between *T. rhodesiense* and *T. gambiense* has already been noted by Stephens and Fantham (1910).

(6) It follows, especially from the incubation period and the duration of life in sub-inoculated rats, that the Rhodesian strain is the more virulent. The difference of virulence is more markedly shown in the duration of life of infected guinea-pigs (see p. 457).

Curiously enough, we find that the incubation period does not seem to be affected so much by the dose (number of trypanosomes) inoculated as by the resistance of the animals. (Cf. Professor Ross's 'Prevention of Malaria' (1910), pp. 94, 95.)

ENUMERATIONS IN GUINEA-PIGS

Exactly the same methods as already set forth were followed in the counts of parasites in guinea-pigs. The same two strains of trypanosomes were again used, five animals being inoculated with each strain. We append tables of our results, together with typical charts.

1. *T. rhodesiense*

GUINEA-PIG 1.—Weight 346 grams. Dose of inoculation: 500,000 Trypanosomes. Incubation period 6 days. Duration of life 79 days. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	4
Leucocytes	—	6,000	7,680	—	13,000	6,720	3,884
Temp.	—	80	84	76	—	70	76
Weight in grams	—	346	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	28	44	20	8	48	2	4
Leucocytes	18,944	11,328	13,440	4,920	26,400	10,780	9,408
Temp.	75	74	70	78	—	74	80
Weight in grams	—	—	—	—	—	—	—

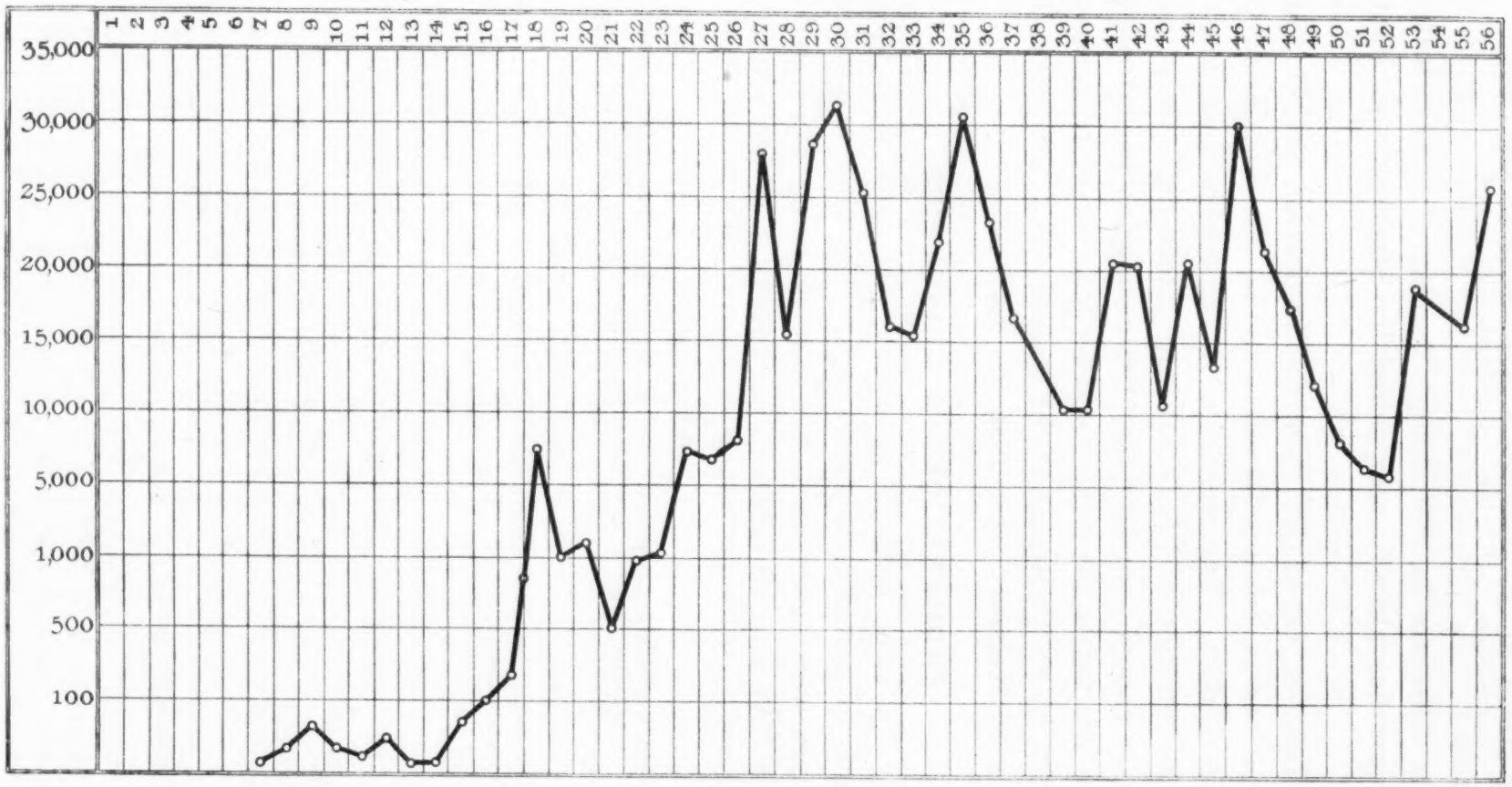


CHART 4.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. rhodesiense*. Periodic Variation. (See record of Guinea-pig 1.)

Guinea-pig 1—continued.

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	68	104	216	8,712	1,010	1,936	504
Leucocytes	—	—	—	—	—	—	—
Temp.	80	80	80	66	—	76	80
Weight in grams	—	408	—	—	—	—	—

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	1,152	1,360	8,576	8,064	9,936	28,224	15,504
Leucocytes	—	4,200	24,384	14,400	24,360	—	—
Temp.	76	76	74	76	—	80	81
Weight in grams	—	—	—	—	—	—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	29,488	32,320	25,200	16,800	15,792	23,200	30,944
Leucocytes	—	—	—	—	—	—	—
Temp.	80	82	80	80	—	—	94
Weight in grams	—	425	—	479	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	23,688	16,800	—	10,560	10,520	21,280	20,208
Leucocytes	—	—	—	—	—	—	—
Temp.	81	83	—	—	—	82	—
Weight in grams	—	—	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	11,172	21,334	14,784	30,448	24,812	17,952	12,960
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 1—continued.

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	9,920	8,920	7,448	19,824	—	17,612	26,496
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

The animal lived for 23 days after the last count was made.

The graph of Guinea-pig 1 is shown in Chart 4.

GUINEA-PIG 2, ♂.—Weight 880 grams when inoculated; 614 grams at death. Dose of inoculation: 500,000 Trypanosomes. Duration of life 28 days. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	2	0	1	4	4	12
Temp.		12	70	66	26		65	62

Day	9	10	11	12	13	14	15
Parasites per c.mm.	8	100	150	1,000	896	1,680	7,992
Temp.	74	70	64	66		76	68

Day	16	17	18	19	20	21	22
Parasites per c.mm.	6,000	5,632	7,200	21,600	45,600	25,000	11,340
Temp.	63	75	80	70	73	52	20

Day	23	24	25	26	27	28	
Parasites per c.mm.	6,720	4,416	18,720	9,600	6,000	30,720	Died
Temp.	80	82	10	64	78	6	

GUINEA-PIG 3, ♂.—Weight 952 grams at time of inoculation; 880 grams at death. Dose of inoculation: two million Trypanosomes. Duration of life 82 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	8	4
Temp.		25				20	

Day		46	47	48	49	50
Parasites per c.mm.	Interval of 38 days' vacation	12,600	80,064	68,448	23,562	9,972
Temp.		32	44	40	38	34

Day	51	52	53	54	55	56	57
Parasites per c.mm.	4,840	2,320	6,040	7,200	3,600	2,560	4,284
Temp.		44	30	40	68	26	28

Day	58	59	60	61	62	63	64
Parasites per c.mm.	8,600	12,480	23,296	3,328	4,320	1,600	3,072
Temp.		40	40	41	34	30	34

Day	65	66	67	68	69	70	71
Parasites per c.mm.	6,400	8,640	1,280	2,080	1,024	768	1,200
Temp.		46	30	44	40	42	43

Day	72	73	74	75	76	77	78
Parasites per c.mm.	10,560	6,048	5,880	2,480	4,320	5,760	4,032
Temp.		38	40	32	32	33	26

Guinea-pig 3—continued.

Day	79	80	81	82		—	—
Parasites per c.mm.	1,296	720	140	6,000	Died	—	—
Temp.	24	40	36	14		—	—

Guinea-pig 4, ♂.—Weight 440 grams at time of inoculation; 402 grams at time of death. Dose of inoculation: two million Trypanosomes. Duration of life 57 days. (H.B.F.)

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	1,920	8	8	0
Temp.				13	22	31	22	33

Day		47	48	49	50	51
Parasites per c.mm.	Interval of 38 days' vacation	10,000	67,392	113,568	33,880	96,100
Temp.		20	24	46	32	

Day	52	53	54	55	56	57	
Parasites per c.mm.	50,000	26,740	14,864	7,168	3,024	24,720	Died
Temp.	40	36	36	49	34	2	

Guinea-pig 5, ♀.—Weight 492 grams when inoculated; 447 grams at death. Dose of inoculation: 1½ million Trypanosomes. Duration of life 51 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Temp.			30				

Day	8	9	10	11	12	13	14
Parasites per c mm.	—	—	—	12	4	56	40
Temp.	44	40	48	48	46	40	

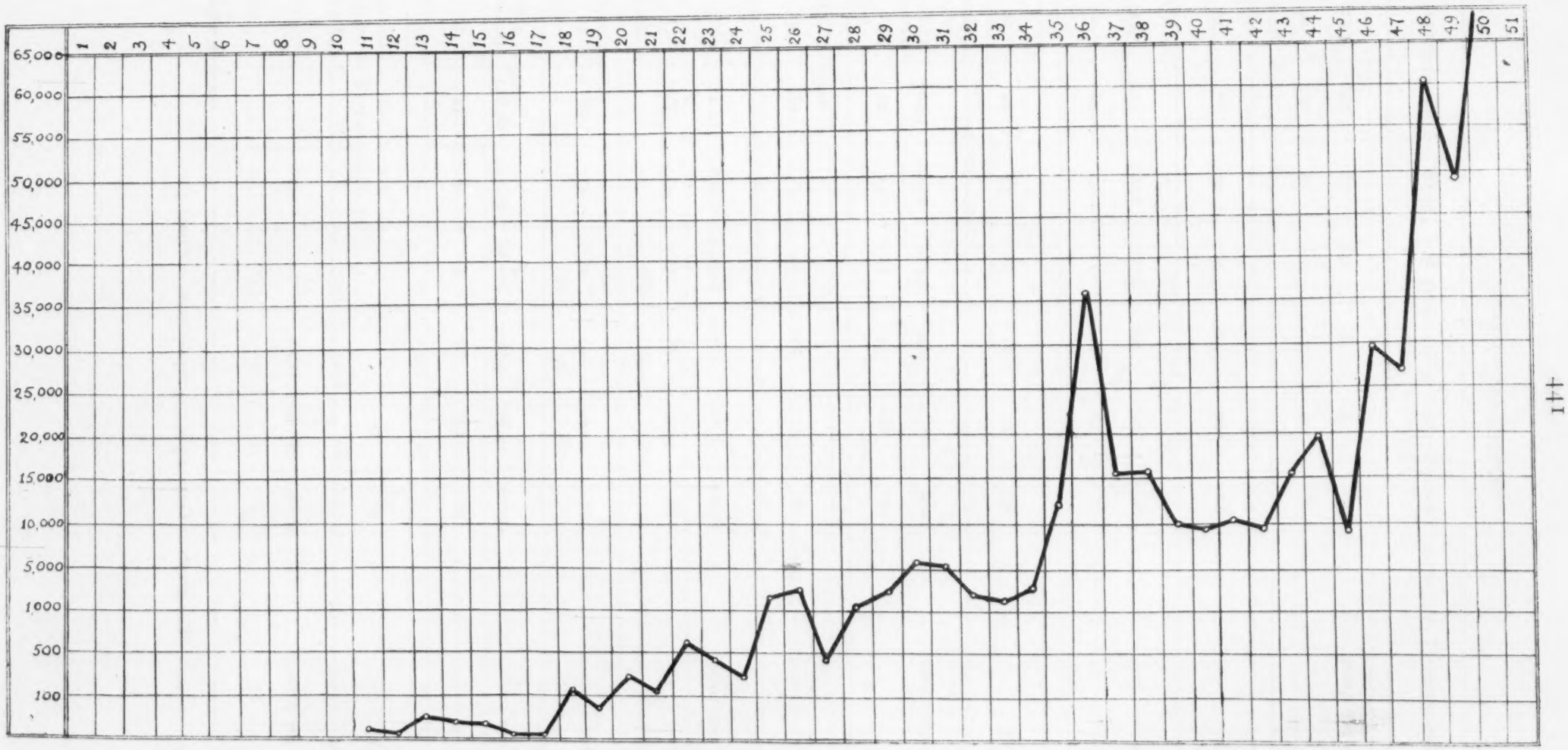


CHART 5.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. rhodesiense*. Periodic Variation. (See record of Guinea-pig 5.)

Guinea-pig 5—continued.

Day	15	16	17	18	19	20	21
Parasites per c.mm.	36	4	4	148	76	240	120
Temp.	36	52	30	46	34	30	24

Day	22	23	24	25	26	27	28
Parasites per c.mm.	660	420	260	2,700	3,264	432	1,024
Temp.	40	26	12	12	25	26	

Day	29	30	31	32	33	34	35
Parasites per c.mm.	3,200	6,400	5,440	2,744	2,352	3,520	12,800
Temp.	23	38	24	38	45	20	

Day	36	37	38	39	40	41	42
Parasites per c.mm.	35,200	15,680	15,760	10,000	9,408	10,240	9,800
Temp.	50	47	38	40	40	36	

Day	43	44	45	46	47	48	49
Parasites per c.mm.	15,120	19,600	9,600	29,568	27,320	60,624	49,140
Temp.	50	44	23	30	28	54	50

Day	50	51					
Parasites per c.mm.	96,880	164,160	Died	—	—	—	—
Temp.	38	0		—	—	—	—

The graph of Guinea-pig 5 is represented in Chart 5.

2. *T. gambiense*

GUINEA-PIG 6.—Weight 403 grams. Dose of inoculation: 4,000,000 Trypanosomes. Duration of life 79 days. (J.G.T.)

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	12	16	24	8	0
Leucocytes	—	—	—	—	—	—	—
Temp.	78	73	91	82	84	70	77
Weight in grams	403	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	8	8	4	0	8	76	48
Leucocytes	—	—	—	—	—	—	—
Temp.	76	85	86	—	90	80	91
Weight in grams	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	680	1,440	960	—	272	440	416
Leucocytes	—	—	—	—	—	—	—
Temp.	80	76	80	—	74	76	88
Weight in grams	405	—	—	—	—	—	—

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	1,296	420	480	270	2,552	1,320	6,000
Leucocytes	—	—	—	—	—	—	—
Temp.	81	90	82	—	—	72	80
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 6—continued.

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	13,392	21,620	29,160	44,016	28,836	14,812	13,396
Leucocytes	—	—	13,428	—	16,200	7,452	7,548
Temp.	74	—	86	—	84	80	80
Weight in grams	427	—	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	29,592	39,720	52,600	39,396	27,840	67,032	113,160
Leucocytes	8,856	11,200	12,400	5,964	—	7,068	—
Temp.	70	76	70	—	82	76	82
Weight in grams	—	—	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	88,000	100,400	45,080	110,400	60,904	40,240	55,240
Leucocytes	6,920	8,320	5,100	14,812	6,888	6,240	7,920
Temp.	82	82	80	—	70	78	80
Weight in grams	538	—	—	—	—	—	—

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	32,544	99,000	71,680	75,674	74,784	74,112	61,600
Leucocytes	4,032	8,530	—	—	—	—	—
Temp.	81	84	86	—	86	78	78
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 6—continued.

Day	57	58	59	60	61	62	63
Number of Trypanosomes per c.mm.	67,926	85,184	26,460	56,280	33,600	70,752	80,726
Leucocytes	—	—	9,976	—	—	—	—
Temp.	76	76	78	—	—	99	80
Weight in grams	574	—	—	—	—	—	—

Day	64	65	66	67	68	69	70
Number of Trypanosomes per c.mm.	64,000	50,232	27,456	44,000	63,962	91,056	72,432
Leucocytes	—	—	—	—	—	19,152	—
Temp.	80	—	—	—	76	—	—
Weight in grams	610	—	—	—	—	—	—

Day	71	72	73	74	75	76	77
Number of Trypanosomes per c.mm.	57,624	62,800	72,384	100,128	62,496	75,520	49,856
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Day	78	79	—	—	—	—	—
Number of Trypanosomes per c.mm.	80,808	53,176	Died	—	—	—	—
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

GUINEA-PIG 7.—Incubation period 4 days. Duration of life 105 days. (J.G.T.)

Day	20	21	22	23	24	25	26
Number of Trypanosomes per c.mm.	30,190	15,120	6,784	4,280	3,852	30,400	42,008
Temp.	—	86	80	76	83	89	92
Weight in grams	—	—	—	534	—	—	—
Leucocytes	—	—	—	—	—	—	12,000

Day	27	28	29	30	31	32	33
Number of Trypanosomes per c.mm.	32,212	28,120	7,914	18,698	13,860	23,440	21,720
Temp.	86	80	85	84	82	85	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	34	35	36	37	38	39	40
Number of Trypanosomes per c.mm.	8,040	13,784	8,460	9,720	9,600	32,572	—
Temp.	84	107	90	80	76	78	—
Weight in grams	—	—	—	467	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	41	42	43	44	45	46	47
Number of Trypanosomes per c.mm.	16,992	10,000	3,840	432	1,760	1,940	320
Temp.	82	80	82	84	84	86	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

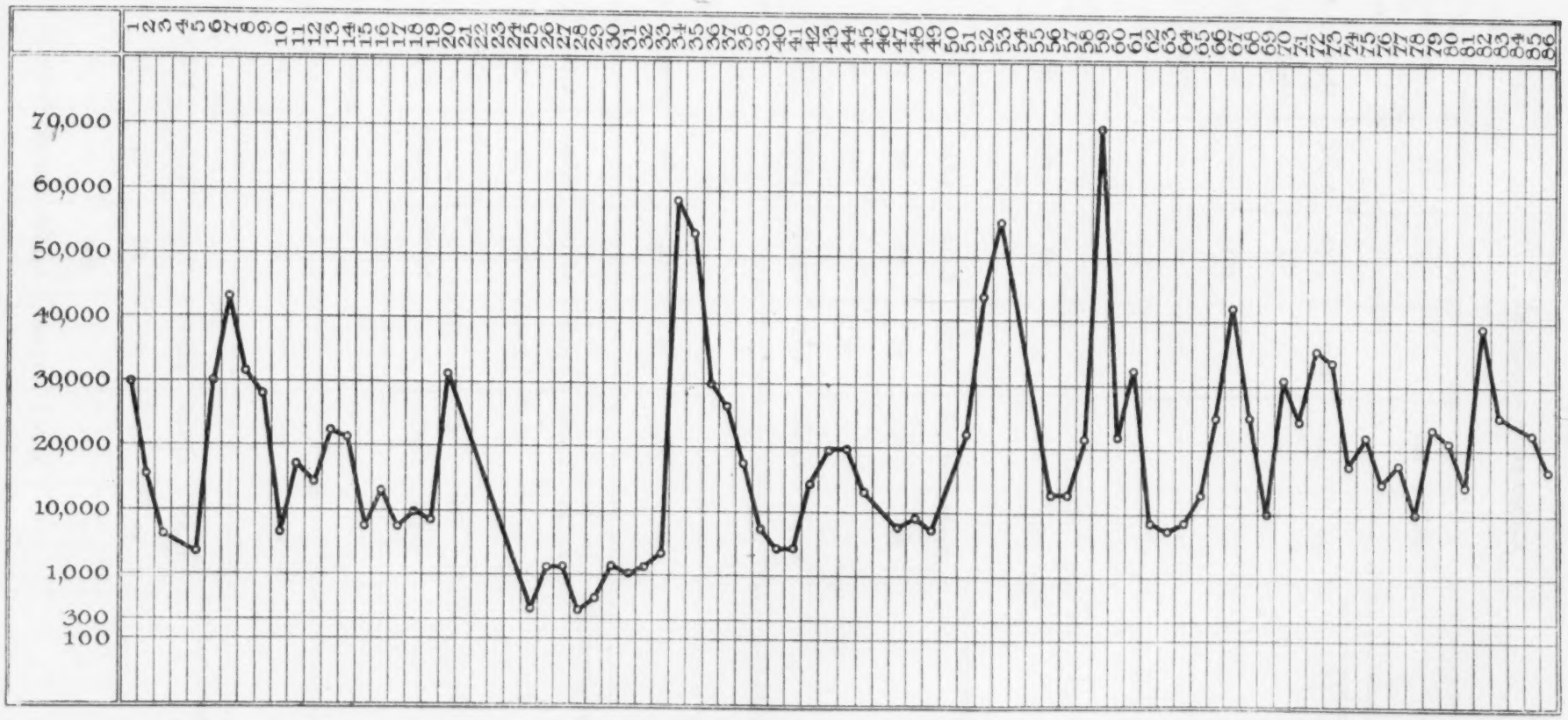


CHART 6.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. gambiense*. Periodic Variation. (See record of Guinea-pig 7.)

[The graph represents the daily numbers of the parasites from the 20th to the 105th day, when death occurred. The days should have been numbered accordingly, and not as 1 to 86.]

Guinea-pig 7—continued.

Day	48	49	50	51	52	53	54
Number of Trypanosomes per c.mm.	552	1,440	1,000	1,232	3,484	58,608	52,384
Temp.	—	78	76	76	—	72	—
Weight in grams	—	—	—	463	—	—	—
Leucocytes	—	—	—	—	—	17,160	26,784

Day	55	56	57	58	59	60	61
Number of Trypanosomes per c.mm.	31,124	26,416	18,368	8,112	5,840	5,184	15,656
Temp.	80	78	75	74	74	76	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	10,408	12,480	24,832	23,972	15,920	31,644	22,116

Day	62	63	64	65	66	67	68
Number of Trypanosomes per c.mm.	19,244	19,584	13,760	11,360	8,640	9,502	7,776
Temp.	73	78	80	80	80	70	76
Weight in grams	—	—	—	—	—	—	—
Leucocytes	18,564	18,432	—	26,960	20,480	41,272	—

Day	69	70	71	72	73	74	75
Number of Trypanosomes per c.mm.	15,800	23,848	42,480	57,240	45,800	30,100	12,144
Temp.	77	80	80	86	84	80	80
Weight in grams	—	—	—	500	—	—	—
Leucocytes (local)	—	12,672	25,040	15,480	14,560	—	—

Guinea-pig 7—continued.

Day	76	77	78	79	80	81	82
Number of Trypanosomes per c.mm.	12,180	21,328	70,000	21,160	32,944	9,048	7,392
Temp.	80	80	80	86	84	84	84
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	83	84	85	86	87	88	89
Number of Trypanosomes per c.mm.	9,056	12,760	25,632	42,000	25,000	10,570	31,680
Temp.	—	86	85	80	—	—	—
Weight in grams	—	—	—	513	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	90	91	92	93	94	95	96
Number of Trypanosomes per c.mm.	24,180	35,112	34,368	17,248	21,648	13,832	17,920
Temp.	82	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	97	98	99	100	101	102	103
Number of Trypanosomes per c.mm.	10,248	23,832	21,888	13,072	39,128	25,576	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	104	105	—	—	—	—	—
Number of Trypanosomes per c.mm.	23,056	17,848	Died	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

The graph of Guinea-pig 7 is represented in Chart 6.

GUINEA-PIG 8, ♂.—Weight 867 grams at time of inoculation; 830 grams on 92nd day; 755 grams at death. Dose of inoculation: 500,000 Trypanosomes. Duration of life 130 days. (H.B.F.)

Day	1	2	3	4	5	6	
Parasites per c.mm.	—	—	—	—	8	4	Interval of 38 days' vacation
Temp.		30			35	36	

Day	45	46	47	48	49	50	51
Parasites per c.mm.	7,540	8,640	5,808	13,156	14,400	18,000	1,280
Temp.	26	16	20	44	20	55	20

Day	52	53	54	55	56	57	58
Parasites per c.mm.	4.896	1,792	576	11,000	9,600	12,800	18,200
Temp.	34	46	30	33	38	50	46

Day	59	60	61	62	63	64	65
Parasites per c.mm.	9,216	1.024	2,916	6,000	7,680	10,000	2,000
Temp.	36	54	36	20	34	60	40

Day	66	67	68	69	70	71	72
Parasites per c.mm.	1,220	1,440	720	1,624	2,160	5,600	11,520
Temp.	30	46	26	50	52	26	64

Day	73	74	75	76	77	78	79
Parasites per c.mm.	6,624	4,480	5,760	3,600	5,760	2,016	2,400
Temp.	34	40	50	46	54	30	60

Guinea-pig 8—continued.

Day	80	81	82	83	84	85	86
Parasites per c.mm.	6,600	5,600	8,400	12,000	4,032	1,920	2,304
Temp.	20	24	42	36	46	76	72

Day	87	88	89	90	91	92	93
Parasites per c.mm.	2,184	2,160	3,920	7,840	4,608	3,360	5,040
Temp.	68	69	66	64	64	68	70

Day	94	95	96	97	98	99	100
Parasites per c.mm.	3,920	4,200	6,400	5,960	2,268	2,160	5,760
Temp.	84	64	70	62	60	64	62

Day	101	102	103	104	105	106	107
Parasites per c.mm.	15,680	19,584	9,792	18,000	36,960	5,400	9,600
Temp.	58	56	54	70	74	72	66

Day	108	109	110	111	112	113	114
Parasites per c.mm.	18,000	17,920	14,400	16,000	19,600	8,640	15,680
Temp.	72	74	66	70	76	72	80

Day	115	116	117	118	119	120	121
Parasites per c.mm.	23,040	5,600	9,600	10,240	6,400	12,800	10,240
Temp.	64	74	70	56	76	60	70

Guinea-pig 8—continued.

Day	122	123	124	125	126	127	128
Parasites per c.mm.	16,800	9,600	16,364	25,088	864	7,200	576
Temp.	70	80	74	88	74	82	74

Day	129	130	—	—	—	—	—
Parasites per c.mm.	900	15,000	Died	—	—	—	—
Temp.	86	0		—	—	—	—

GUINEA-PIG 9, ♀.—Weight 529 grams at time of inoculation; 440 grams at death. Dose of inoculation: 100,000 Trypanosomes. Duration of life 96 days. (H.B.F.)

Day	1	2	3	4	5	6	
Parasites per c.mm.	—	—	—	—	4	8	Interval of 38 days' vacation
Temp.		30			24	35	

Day	45	46	47	48	49	50	51
Parasites per c.mm.	28	36	84	72	228	240	256
Temp.	32	20	31	24	22		25

Day	52	53	54	55	56	57	58
Parasites per c.mm.	396	1,820	720	1,350	7,500	7,840	10,920
Temp.	30	20	20	28	38	20	23

Day	59	60	61	62	63	64	65
Parasites per c.mm.	15,340	10,368	7,680	8,064	15,680	16,800	18,400
Temp.	41	26	38	32	32	12	40

Guinea-pig 9—continued.

Day	66	67	68	69	70	71	72
Parasites per c.mm.	3,864	7,920	3,600	1,700	2,880	5,840	8,400
Temp.	30	35	30	25	30		44

Day	73	74	75	76	77	78	79
Parasites per c.mm.	12,800	9,600	7,200	3,000	8,640	9,200	8,400
Temp.	40	30	28	40	34	36	40

Day	80	81	82	83	84	85	86
Parasites per c.mm.	67,648	59,860	36,800	27,000	10,080	5,880	3,072
Temp.	30	20	26	42	20	70	74

Day	87	88	89	90	91	92	93
Parasites per c.mm.	5,120	7,840	3,600	6,272	5,400	6,720	25,600
Temp.	72	70	44	56	62	70	5

Day	94	95	96	—	—	—	—
Parasites per c.mm.	14,400	11,200	42,000	Died	—	—	—
Temp.	26	30	0	—	—	—	—

Guinea-pig 10, ♀.—Weight 495 grams at time of inoculation; 402 grams on 100th day; 335 grams at death. Dose of inoculation: two million Trypanosomes. Duration of life 147 days. (H.B.F.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Temp.	50		45			50	

Guinea-pig 10—continued.

Day	8	9	10	11	12	13	14
Parasites per c.mm.	4	0	0	52	4	4	16
Temp.	56	26	46	50	40	50	

Day	15	16	17	18	19	20	21
Parasites per c.mm.	200	16	4	4	4	12	8
Temp.	34	50	40	31	26	13	22

Day	22	23	24	25	26	27	28
Parasites per c.mm.	4	56	8	4	4	24	4
Temp.	23	44	20	24	28		20

Day	29	30	31	32	33	34	35
Parasites per c.mm.	4	4	8	4	20	20	8
Temp.	27	18	50	51	20	53	40

Day	36	37	38	39	40	41	42
Parasites per c.mm.	4	4	16	8	76	8	8
Temp.	36	36	31	34		26	34

Day	43	44	45	46	47	48	49
Parasites per c.mm.	0	0	0	0	24	20	480
Temp.	34	30	38	34	32	26	

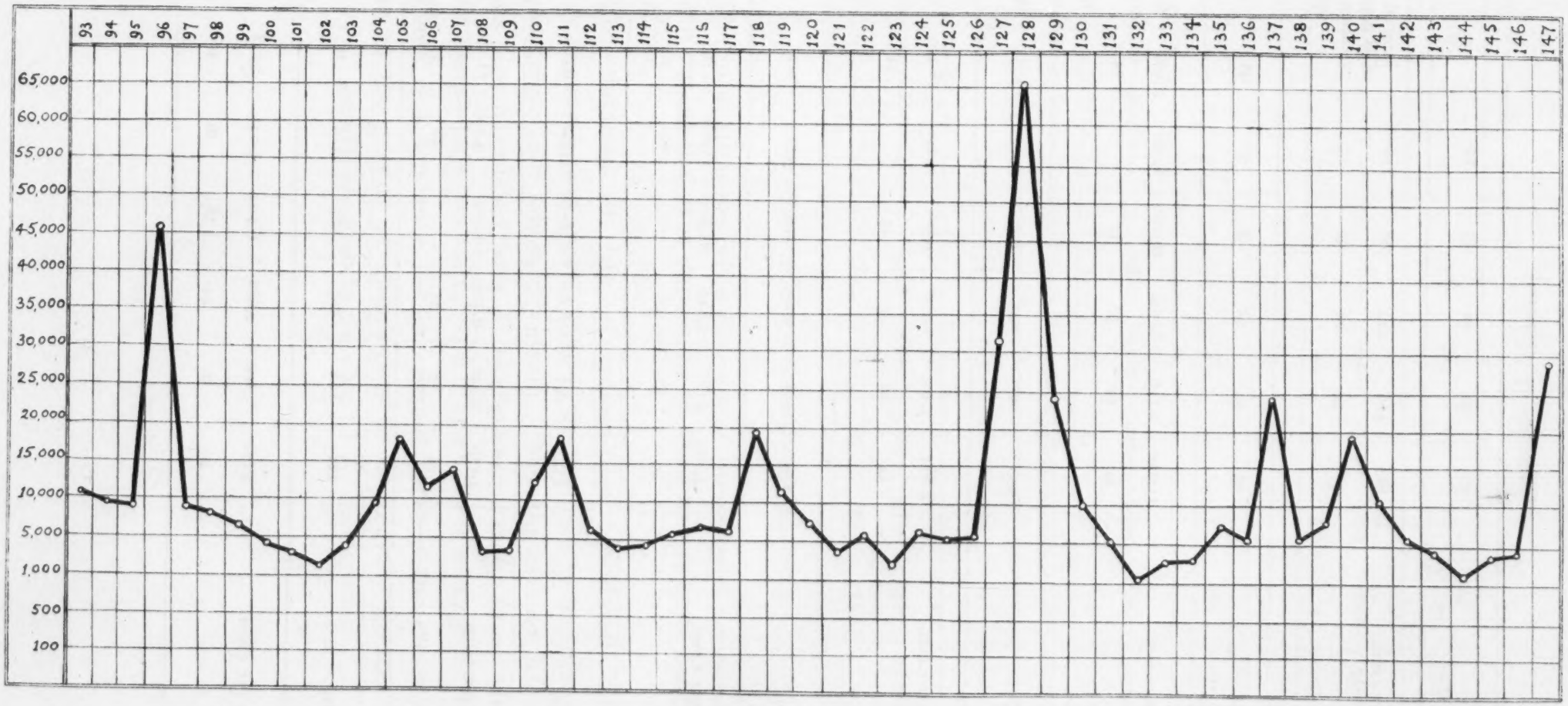


CHART 7.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Guinea-pig inoculated with *T. gambiense*. Periodic Variation. (See record of Guinea-pig 10.)

Guinea-pig 10—continued.

Day	50	51	52	53	54		
Parasites per c.mm.	1,880	416	124	368	116	Interval of 38 days' vacation	
Temp.	40	34	23	32	15		
Day	93	94	95	96	97	98	99
Parasites per c.mm.	11,240	9,216	8,064	46,180	8,096	7,920	6,192
Temp.	34	44	30	32		34	42
Day	100	101	102	103	104	105	106
Parasites per c.mm.	4,864	3,300	2,000	4,576	9,240	18,200	12,480
Temp.	34	46	30	32		51	31
Day	107	108	109	110	111	112	113
Parasites per c.mm.	14,400	3,168	3,840	12,800	18,600	6,160	3,920
Temp.	20	50	42		60	50	52
Day	114	115	116	117	118	119	120
Parasites per c.mm.	4,536	5,040	6,912	6,000	19,200	12,000	7,020
Temp.	50	32	25		48	35	22
Day	121	122	123	124	125	126	127
Parasites per c.mm.	3,520	5,600	2,688	6,400	5,040	5,728	32,000
Temp.	40	30	44	38	36	40	40

Guinea-pig 10—continued.

Day	128	129	130	131	132	133	134
Parasites per c.mm.	66,000	24,500	10,000	5,040	1,008	3,600	3,888
Temp.	30	22	40	33	54	44	63

Day	135	136	137	138	139	140	141
Parasites per c.mm.	7,680	5,760	24,000	5,760	7,680	19,440	11,880
Temp.	44	70	44	70	78	72	96

Day	142	143	144	145	146	147	—
Parasites per c.mm.	6,480	4,860	2,268	3,888	4,480	28,880	Died
Temp.	80	76	30	52	14	0	

The graph of Guinea-pig 10 is represented in Chart 7.

The incubation period in the case of guinea-pigs varies so much in both strains that it is impossible to draw any definite conclusions therefrom.

The duration of life of guinea-pigs inoculated with the Rhodesian strain was shorter than in those similarly inoculated with *T. gambiense* (old laboratory strain); thus, the average life of the guinea-pigs inoculated with *T. rhodesiense* was 59 days, while the life of guinea-pigs inoculated with *T. gambiense* averaged 111 days.

There is a tendency for the period between the crests of the waves in the graphs of guinea-pigs to be slightly greater in the case of *T. gambiense* than in the case of *T. rhodesiense*.

Guinea-pig 10—continued.

Day	50	51	52	53	54		
Parasites per c.mm.	1,880	416	124	368	116	Interval of 38 days' vacation	
Temp.	40	34	23	32	15		
Day	93	94	95	96	97	98	99
Parasites per c.mm.	11,240	9,216	8,064	46,180	8,096	7,920	6,192
Temp.	34	44	30	32		34	42
Day	100	101	102	103	104	105	106
Parasites per c.mm.	4,864	3,300	2,000	4,576	9,240	18,200	12,480
Temp.	34	46	30	32		51	31
Day	107	108	109	110	111	112	113
Parasites per c.mm.	14,400	3,168	3,840	12,800	18,600	6,160	3,920
Temp.	20	50	42		60	50	52
Day	114	115	116	117	118	119	120
Parasites per c.mm.	4,536	5,040	6,912	6,000	19,200	12,000	7,020
Temp.	50	32	25		48	35	22
Day	121	122	123	124	125	126	127
Parasites per c.mm.	3,520	5,600	2,688	6,400	5,040	5,728	32,000
Temp.	40	30	44	38	36	40	40

Guinea-pig 10—continued.

Day	128	129	130	131	132	133	134
Parasites per c.mm.	66,000	24,500	10,000	5,040	1,008	3,600	3,888
Temp.	30	22	40	33	54	44	63

Day	135	136	137	138	139	140	141
Parasites per c.mm.	7,680	5,760	24,000	5,760	7,680	19,440	11,880
Temp.	44	70	44	70	78	72	96

Day	142	143	144	145	146	147	—
Parasites per c.mm.	6,480	4,860	2,268	3,888	4,480	28,880	Died
Temp.	80	76	30	52	14	0	

The graph of Guinea-pig 10 is represented in Chart 7.

The incubation period in the case of guinea-pigs varies so much in both strains that it is impossible to draw any definite conclusions therefrom.

The duration of life of guinea-pigs inoculated with the Rhodesian strain was shorter than in those similarly inoculated with *T. gambiense* (old laboratory strain); thus, the average life of the guinea-pigs inoculated with *T. rhodesiense* was 59 days, while the life of guinea-pigs inoculated with *T. gambiense* averaged 111 days.

There is a tendency for the period between the crests of the waves in the graphs of guinea-pigs to be slightly greater in the case of *T. gambiense* than in the case of *T. rhodesiense*.

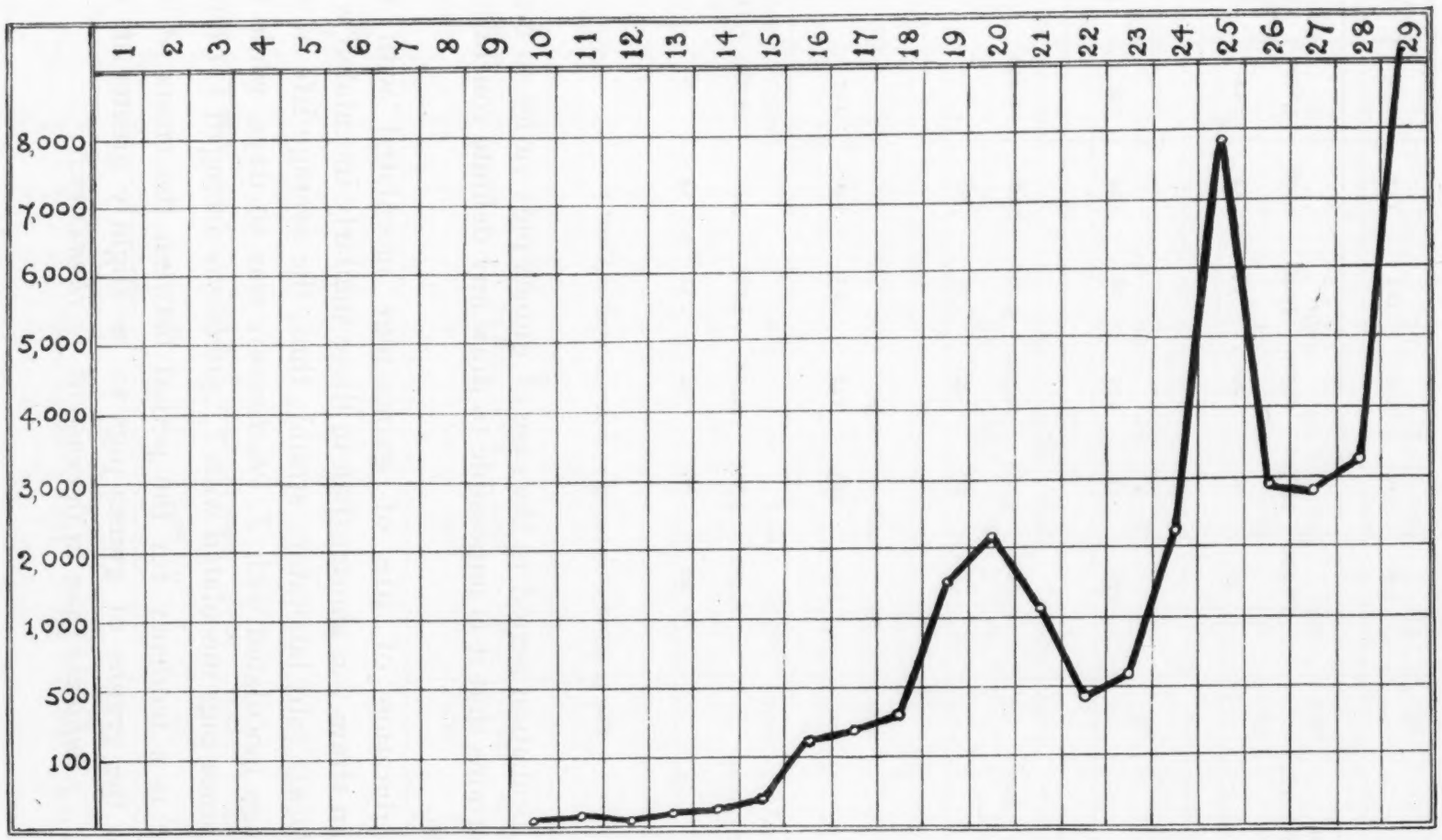


CHART 8.—Graph of Daily Counts of the Parasites in the Peripheral Blood of a Rabbit inoculated with *T. rhodesiense*. Periodic Variation. (See record of Rabbit 1.)

ENUMERATIONS IN RABBITS

Two rabbits were inoculated with *T. rhodesiense* and the course of the infection was followed in them, the same methods being employed as in previous cases. We append tables:—

T. rhodesiense

RABBIT 1.—Grey and white. Dose of inoculation: 600,000 Trypanosomes, intraperitoneally. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Parasites per c.mm.	—	—	2	4	1	6	8

Day	15	16	17	18	19	20	21
Parasites per c.mm.	32	168	200	360	1,500	2,184	1,008

Day	22	23	24	25	26	27	28
Parasites per c.mm.	460	672	2,200	7,960	2,944	2,880	3,360

Day	29	—	—	—	—	—	—
Parasites per c.mm.	35,200	Died	—	—	—	—	—

The graph of Rabbit 1 is shown in Chart 8.

RABBIT 2.—Grey. Dose of inoculation: 600,000 Trypanosomes, intraperitoneally. (J.G.T.)

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	—	—
Day	8	9	10	11	12	13	14
Parasites per c.mm.	—	—	2	8	12	48	2
Day	15	16	17	18	19	20	21
Parasites per c.mm.	15	1	4	20	100	160	420
Day	22	23	24	—	—	—	—
Parasites per c.mm.	1,776	2,320	2,184	Died	—	—	—

COMPARISON OF THE EFFECTS OF THE TRYPANOSOMES ON THE VARIOUS HOSTS

In guinea-pigs the disease tended to run a more chronic course than in rats, as shown by the fact that the animals lived much longer. The periods between the crests of the waves in both strains in guinea-pigs were longer than in rats, namely five to eight days in most cases. Further, in guinea-pigs the initial rise is slower—a 'staircase' effect taking place and leading to the chronic condition (see Charts 4, 5).

Some of the guinea-pigs when the disease became chronic showed an alternation of high and low rises, which simulate remarkably the graph of the parasites of the patient W.A. (see R. Ross and D. Thomson (1910)*).

In the two rabbits used it was remarkable that these were the only animals that exhibited an affection of the eyes and skin. A

* Proc. Roy. Soc., B, Vol. LXXXII, p. 413.

white purulent discharge occurred from the eyes in both rabbits about the seventh day after inoculation. Simultaneously with this affection of the eyes the skin at the roots of the ears became encrusted with scabs. Both rabbits became drowsy early in the disease, and remained so until death. Although the incubation period in the case of rabbits was long (nine days), yet the animals early succumbed to the effects of the disease, in spite of the fact that the trypanosomes were never very numerous in the peripheral blood.

Rats also showed drowsiness during the latter half of the infection, while guinea-pigs continued to be lively and to take food well until a day or so before death, when in some cases drowsiness ensued.

In our experiments we did not observe any marked relation between the colour of the animal inoculated and the duration of infection in that animal. (It is sometimes thought that white rats are less resistant than piebald.)

In passing it may be noted, as has been done before, that the temperature of the host tended to rise with increase in the number of trypanosomes in the peripheral blood.

EXPLANATION OF THE PERIODIC INCREASE AND DECREASE IN THE NUMBERS OF TRYPANOSOMES IN THE PERIPHERAL BLOOD OF THE HOST

We believe that periodic increase and decrease of the numbers of trypanosomes in the peripheral blood of the host is a natural phenomenon, resulting from the reaction of the host and parasite on each other.

The parasites increase by longitudinal division until a maximum is reached when, unfavourable conditions arising, the formation of rounded, latent, non-flagellate bodies occurs, especially in the internal organs of the host where they collect in the spleen and bone marrow during the period of decrease of flagellates in the peripheral blood. These rounded bodies, or at least some of them, become flagellate trypanosomes when the parasites begin to increase in numbers again (see Fantham (1911)†).

† Proc. Roy. Soc., B, Vol. LXXXIII, pp. 212-227, and Ann. Trop. Med. and Parasitol., Vol. IV, pp. 465-485.

The slight fluctuations in the periodicity, namely, the variations in the time-intervals between the crests of the waves, can be explained by the fact that the resistance of the animal varies from time to time. Again, we might note that the rat, the guinea-pig, and the rabbit are not the natural hosts of the parasites of sleeping sickness.

We should also note that the strains with which we have been dealing are very virulent, as judged by the records of Thomas and Linton (1904),* Moore and Breinl (1907), and others, whose inoculated animals lived much longer than ours. In less virulent strains the time-intervals between the crests of the waves in the graphs might tend to be longer.

The periodic variation or oscillation that we have described is of practical importance in the administration of drugs to eliminate trypanosomes in a host. If the drug be administered on the downward slope of the curve, the fall in numbers of trypanosomes is not due to the drug alone. But if the drug be administered on the upward slope of the curve, while the parasites are increasing in numbers, and yet a reduction in numbers of the parasites is brought about, then the drug may legitimately be inferred to be efficacious.

SUMMARY

1. In these investigations two strains of trypanosomes were used, namely, (*α*) *T. rhodesiense* (Stephens and Fantham) derived from a patient, W.A., suffering from sleeping sickness contracted in Rhodesia, and (*β*) *T. gambiense*, an old laboratory strain.

2. Of these two strains the Rhodesian was the more virulent, as judged by sub-inoculations in rats, guinea-pigs and rabbits, for the duration of life of animals inoculated with the Rhodesian strain was shorter than in those inoculated with *T. gambiense*.

Stephens and Fantham (1910) have already pointed out a distinct morphological peculiarity in *T. rhodesiense* in the presence of a posterior nucleus in some of the stout forms.

3. Enumeration of trypanosomes in the peripheral blood of rats, guinea-pigs and rabbits inoculated with each of these strains

* Lancet, May 14, 1904, pp. 1337-1340.

reveals remarkable variations or oscillations in the numbers of the parasites from day to day, the variations being of the nature of a periodic increase and decrease at more or less regular intervals.

4. The periodicity is to be explained by (α) variations in resistance on the part of the host, probably due to the formation of anti-bodies, accompanied by (β) the formation on the part of the trypanosomes of rounded, latent, non-flagellate (relatively resistant) forms in the internal organs of the host during the fall in numbers of the flagellate parasites in the peripheral blood.

There is a life-cycle of trypanosomes in the Vertebrate host, in addition to stages of the parasite in the Invertebrate carrier (for example, *Glossina*).

THE LIFE-HISTORY OF *TRYPANOSOMA GAMBIENSE* AND *TRYPANOSOMA RHODESIENSE* AS SEEN IN RATS AND GUINEA-PIGS*

BY

H. B. FANTHAM, D.Sc. Lond., B.A. Cantab., A.R.C.S.

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PLATE XXVII.

CONTENTS

INTRODUCTION

The researches recorded in this paper were undertaken at the suggestion of Major Ross, who wished me to investigate the parasitological aspect of the numerical cyclical development discovered by him and Dr. D. Thomson (1910) in the trypanosome occurring in a patient suffering from sleeping sickness contracted in Rhodesia, particularly as regards the possible connection of the latent bodies of Salvin-Moore and Breinl (1907) with that cycle. The investigations have been conducted in the Liverpool School of Tropical Medicine, under a grant from the Tropical Diseases Research Fund.

* Read before the Royal Society on December 8, 1910, and reprinted from Proc. Roy. Soc., B, Vol. LXXXIII, pp. 212-227.

A complete and generally accepted life cycle of *Trypanosoma gambiense* has yet to be written. The following paper is offered as a contribution to the solution of this difficult problem, and deals with that portion of the life history of the parasite which takes place in a vertebrate host.

The sub-inoculations in animals—rats and guinea-pigs—recorded herein were made from a patient suffering from Rhodesian sleeping sickness in Professor Ross's clinic in the Royal Southern Hospital, Liverpool. The trypanosome from this source showed a marked morphological feature in the possession of a posterior nucleus in some forms, and for this parasite the name *T. rhodesiense* has been suggested by Stephens and Fantham (1910). A laboratory strain of *T. gambiense* was also used in these investigations for comparison.

Special attention has been paid to the observation of the *living* parasite, as well as to stained preparations.

METHODS

Fresh preparations of *T. gambiense* and *T. rhodesiense* were made from blood taken aseptically from animals, namely, tame rats and guinea-pigs. The blood was kept in sealed cover-slip preparations, at or below blood heat (25° to 37° C.), and also at laboratory temperature. The blood was sometimes diluted with a little physiological salt solution or with a little isotonic sodium citrate solution. Methylene blue was sometimes used for *intra vitam* staining.

Wet preparations of the parasite were made on cover-slips after fixation with osmic acid vapour or corrosive acetic alcohol. The chief stains used were iron haematoxylin and those of Giemsa and Romanowsky.

Dry smears were employed for rough work.

In animals killed at certain stages of infection, the various internal organs were carefully examined both in fresh preparations and stained smears.

The enumerative methods used were those recently employed by R. Ross and D. Thomson and used by various workers in Liverpool, being an elaboration of R. Ross's thick-film method made with measured quantities of blood (1 cubic millimetre divided

into quarters). The films were dehaemoglobinised, fixed in absolute alcohol, and stained by the Romanowsky method.

RÉSUMÉ OF PREVIOUS WORK ON LATENT BODIES OF TRYPANOSOMES

The various flagellate forms of *Trypanosoma gambiense* have been so often described by many competent workers that it is needless to discuss them further in detail. Suffice it to say that long, thin trypanosomes may occur, especially at the beginning of infection in rats and guinea-pigs, and shorter, stout or stumpy forms later. Trypanosomes intermediate in character also are found.

The so-called rounded, latent or encysted forms, which are non-flagellate, must be discussed in greater detail. Marked attention to these non-flagellate forms of trypanosome was first drawn by Moore and Breinl (1907-8) in mammalian trypanosomes, though Dutton saw rounded forms of an amphibian trypanosome on the Congo in 1903-5. Moore and Breinl (1907), working on stained material, stated that latent forms occurred in the internal organs, especially during the periods when the flagellates were decreasing or absent from the peripheral blood of the host. These authors give a curve showing the variations in the numbers of *T. gambiense* in infected rats; they do not, however, give any numerical data in support of the graph. A few other workers have mentioned rounded bodies in connection with trypanosomes, among whom Hindle (1909) may be noted. The significance of the latent bodies is still a disputed point, and it was recently stated in a review that more evidence was required to show that they 'constitute part of a life-cycle in the vertebrate host.'*

In the present paper the general statements of Moore and Breinl, regarding forms of *T. gambiense* in the internal organs of rats, are shown to be accurate, and many details, as seen in the living parasite, as well as numerical data from daily counts of the tryparosomes, are supplied in proof of the significance of the latent bodies. Animals inoculated with *T. rhodesiense* (Stephens and Fantham) have also been carefully investigated.

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* S.S. Bureau, Bulletin No. 15 (March, 1910), p. 102.

THE RELATION BETWEEN LATENT BODIES AND THE NUMBER OF TRYPANOSOMES IN THE PERIPHERAL BLOOD, WITH NOTES ON THE PARASITES FOUND IN THE INTERNAL ORGANS OF THE HOSTS

Following on the work of R. Ross and D. Thomson (1910) on the enumeration of the parasites in the peripheral blood of a patient suffering from Rhodesian sleeping sickness, the periodic increase in the number of trypanosomes in the peripheral blood of rats and guinea-pigs inoculated with *T. rhodesiense* or with *T. gambiense* has recently been found by Fantham and J. G. Thomson.

Rats similarly inoculated and exhibiting such periodic increase and decrease were killed at various points in the cycle, as set forth in the following tables:—

A. RAT 1.—*T. gambiense*, Laboratory Strain, in Piebald ♂ Rat, weight 150 grams, inoculated with 50,000 trypanosomes.

Day	1	2	3	4	5	6	7	8
Parasites per c.mm.	—	—	—	—	—	—	4	1088
Temp.*		55	46	70		62	57	54

Day	9	10	11	12	13	14	15
Parasites per c.mm.	4392	868	1656	2560	9288	6304	436, killed
Temp.*	60	68	44	46	57	54	61

* The temperature is expressed according to the following convention, as in Major Ross's recent papers on 'Malaria' and 'Trypanosomiasis':—Temp. = (F − 95) × 10, where F is the temperature in degrees Fahrenheit, recorded with a clinical thermometer.

Heart blood of Rat 1 *examined fresh, immediately after killing.*—A few living trypanosomes seen and some (fewer) rounded forms.

Spleen.—Very large; three to seven rounded forms seen in every field of the microscope (2 mm. objective and 2 ocular); no flagellates seen in fresh preparation.

Liver and portal blood.—Living flagellates and rounded bodies seen in about equal numbers (one of each per field).

Lungs.—A few living flagellates seen, four times as many rounding and rounded bodies seen [100 parasites counted in this and subsequent fresh preparations, when possible].

Bone marrow.—Many rounded bodies.

RAT 2.—*T. gambiense*, Laboratory Strain, in White ♀ Rat, weight 128 grams, inoculated with 200,000 trypanosomes.

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	4	684	3640
Temp.	38		40	45	50	54	60

Day	8	9	10	11	12
Parasites per c.mm.	7392	10,000	6320	1840	2200, killed
Temp.	55	58	44	46	48

The internal organs of Rat 2 were in much the same condition as in Rat 1; further details are superfluous.

B. RAT 3.—*T. rhodesiense* in Piebald Rat, ♀, weight 108 grams, inoculated with 350,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.	—	—	172	18,160	42,120	31,040
Temp.	22	20	20	40	56	36

Day	7	8	9	10
Parsites per c.mm.	21,600	12,844	130,000	61,440, killed
Temp.	20	21	15	14

Heart blood, fresh, of Rat 3.—Free trypanosomes seen.

Spleen.—Many rounded bodies; no free trypanosomes seen.

Liver.—A few free trypanosomes seen.

Lungs.—About equal numbers of flagellates and rounded bodies.

Bone marrow.—Rounded bodies.

RAT 4.—*T. rhodesiense* in White Rat, ♂, weight 120 grams, inoculated with 600,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.	—	—	4	896	2880	31,360
Temp.		20	25	38	50	50

Day	7	8	9	10	11
Parasites per c.mm.	51,200	64,000	22,820	12,500	6144, killed
Temp.	48	47	13	20	31

Heart and liver of Rat 4.—Many large trypanosomes seen in fresh preparations, very few rounded bodies.

Lungs.—A few free flagellates, three times as many rounded forms.

Spleen.—A few small flagellate trypanosomes (3 per cent.); many rounded forms.

Kidney blood.—Flagellate trypanosomes.

Bone marrow.—Rounded bodies.

RAT 5.—*T. rhodesiense* in White Rat, ♀, weight 247 grams, inoculated with 120,000 trypanosomes.

Day	1	2	3	4	5	6
Parasites per c.mm.	—	—	—	—	4	12
Temp.			52	38	34	96

Day	7	8	9	10
Parasites per c.mm.	20	296	624	3744, killed
Temp.	86	86	20	66

Flagellate trypanosomes were seen in all the internal organs of Rat 5. A few latent bodies were seen in the lungs, spleen, and bone marrow.

RAT 6.—*T. rhodesiense* in White Rat, ♂, weight 150 grams, inoculated with 500,000 trypanosomes.

Day	1	2	3	4	5	6	7	8	9
Parasites per c.mm. ...	—	—	—	—	72	2056	1968	6420	592, killed
Temp.	38	36	51	56	54	70	70	62	54

Heart blood, fresh, of Rat 6.—Flagellate parasites seen, only three latent bodies found in a fresh preparation.

Liver.—Flagellates 96, and latent bodies 4; in one hour, however, the number of latent bodies in the fresh preparation had increased 16 times (namely to 64), and the flagellates were correspondingly fewer.

Lungs.—About equal numbers of trypanosomes and latent bodies.

Spleen.—Many latent bodies, no free flagellates seen.

Bone marrow.—A few latent bodies, no flagellates seen.

RAT 7.—*T. rhodesiense* in Piebald Rat, ♀, weight 170 grams, inoculated with 1,000,000 trypanosomes. Weight 182 grams at death, but pregnant.

Day	1	2	3	4	5	6
Parasites per c.mm.	—	—	8	5852	27,916	16,468, killed
Temp.	38		62	55	67	66

Heart blood of Rat 7.—Many flagellates, few rounded bodies.

Liver.—Equal numbers of flagellates and rounded bodies.

Lungs.—Three times as many latent bodies as flagellates.

Bone marrow.—Large rounded bodies.

Spleen.—No flagellates seen, but many rounded bodies.

Splenic vein blood.—Twice as many flagellates as rounded bodies.

Mesenteric gland fluid.—Many flagellates.

Lymph.—Rounded bodies and Crithidia-like forms.

Placental blood.—Living flagellates, few rounded bodies.

Thirteen embryos.—Serous fluid contained a very few trypanosomes.

Embryonic liver.—No flagellate trypanosomes seen.

From counts of the number of trypanosomes in the peripheral blood daily, and from examination of carefully prepared smears of organs, it is found that latent bodies are most numerous when the flagellate parasites are few. If inoculated animals be killed at these periods, very few flagellate trypanosomes are found in the spleen and bone marrow (see preceding tables), but many latent bodies are present in those organs, while rounding forms are seen especially in the lungs.

In the peripheral blood, on the upward slope of the curve representing the numbers of the parasites from day to day, the parasites increase in numbers by longitudinal division to a maximum. At or about this period the formation of rounded or latent bodies begins, and takes place especially in the internal organs.

If rounded (latent) bodies, derived from the internal organs of an infected rat, be placed in warm fresh blood drawn from a normal, uninfected rat, then growth of some of the rounded bodies towards the flagellate trypaniform stage can be seen under the microscope, as is detailed in a subsequent section of this paper.

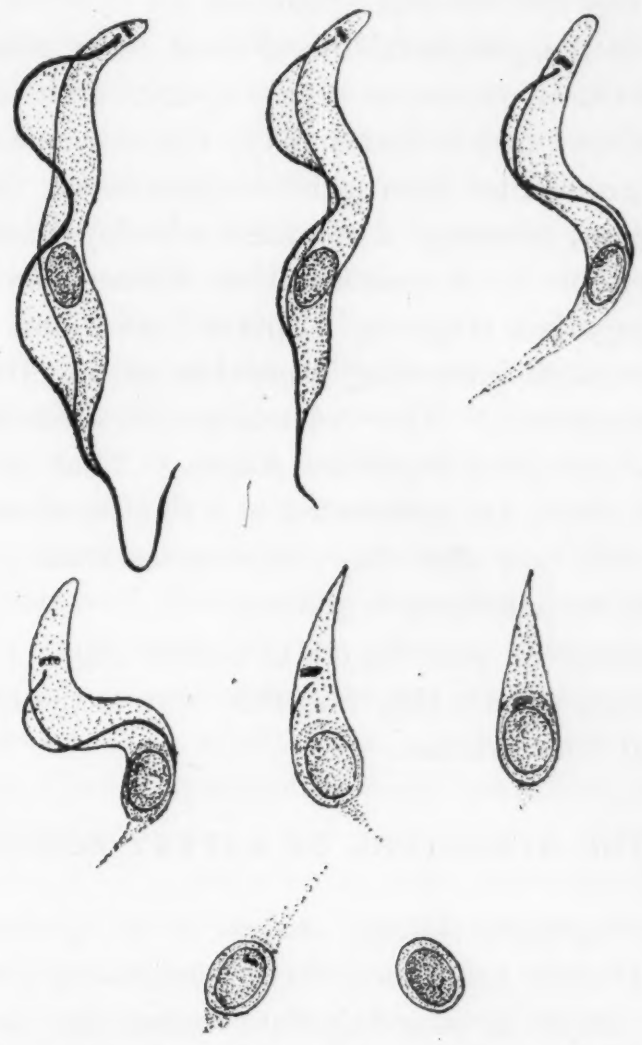
Five guinea-pigs (three inoculated with *T. rhodesiense* and two with *T. gambiense*), dying in various stages of trypanosomiasis, were carefully examined, and fresh preparations and smears of their internal organs were made. Rounding and rounded forms of trypanosomes were seen, just as in infected rats.

Needless to say, a very careful examination of the internal organs of normal (uninfected) rats and guinea-pigs was made for the purposes of comparison and control.

THE FORMATION OF LATENT BODIES FROM FLAGELLATE TRYPANOSOMES

This is diagrammatically represented in text-fig. 1, in which the formation of a rounded body from a living trypanosome was observed under the microscope in a drop of blood and lymph from

the tail of an infected rat. The time taken for the formation of the rounded body was thirty minutes.



TEXT-FIG. 1 represents diagrammatically the formation of a latent (non-flagellate) body of *Trypanosoma gambiense* in infected rat's blood as seen under the microscope, on a warm stage, during a period of 30 minutes.

The intervals were 3, 8, 15, 20, 23, 25, and 30 minutes respectively from the commencement of change of form of the parasite. Intranuclear karyosome not represented.

In this process the anterior or flagellar end disintegrates and is cast off, while at the other end the blepharoplast (kinetonucleus) gradually migrates nearer the nucleus, and then the non-flagellar or posterior end of the original trypanosome is cast off, together with the remains of the flagellum. The rounded body, consisting of chromatin with a thin layer of cytoplasm, has then surrounded itself with a definite, very thin capsule ('cyst'). This process, as seen under the microscope, is either the natural mode of formation of non-flagellate bodies in the internal organs or closely approximates

thereto, as intermediate stages exactly similar in the formation of these bodies are seen in stained preparations of the heart, lungs, and spleen (Plate XXVII, figs. 1-5).

However, in the peripheral blood there occur rounded, oval or somewhat pyriform parasites (figs. 17-20), each with a single anterior flagellum. Such forms may, for convenience, be called rounding herpetomonad forms, as *Herpetomonas* passes through similar stages in assuming a rounded non-flagellate form. The rounded stages of *Herpetomonas*, thus formed, have been aptly termed post-flagellate stages by Captain Patton and by Dr. Annie Porter in their recent interesting researches on flagellates (*Crithidia* and *Herpetomonas*). The rounded, encapsuled stages of trypanosomes are post-flagellate stages. That these rounded, post-flagellate forms are encapsuled in a thin membranous structure is shown by the fact that they resist maceration in water much longer than the trypaniform flagellates.

The formation of post-flagellate bodies (figs. 2, 3, 5) is well seen in the lungs, whence they find their way in the blood stream to the spleen and bone marrow.

THE STRUCTURE OF LATENT BODIES

The post-flagellate (latent) stages of *T. gambiense* and *T. rhodesiense*, already mentioned, have a relatively simple structure. The strictly latent or non-flagellate forms are usually oval in outline, and small, about 2 μ to 4 μ in diameter (figs. 2-16). Less frequently are they quite rounded or spherical (fig. 14), while sometimes they are pyriform (figs. 25, 27, 29, 30). Internally there is a nucleus, which may show a karyosome, and beside the nucleus there is a blepharoplast or kinetonucleus (fig. 6). In some latent bodies, especially the smallest ones, the kinetonucleus may not be visible separately (figs. 11, 13-15), as it may be lying over the nucleus, or actually affixed thereto (figs. 2, 10). The juxtaposition of the nuclear bodies has been actually observed in some Romanowsky-stained specimens, after careful wet fixation (figs. 2, 10). The relative positions of the nucleus and blepharoplast in rounding or rounded bodies may vary considerably. A small quantity of cytoplasm occurs in the latent bodies.

In the formation of rounded, latent bodies, as seen *in vitro*, a portion of the body of the flagellate, after passage of the blepharoplast towards the nucleus, is thrown off, and the flagellum is discarded (text-fig. 1). Examination of preparations of the internal organs (lungs, heart, spleen) of the host shows that a similar method of formation of the post-flagellate stages of the parasite usually occurs in the internal organs (figs. 1-5). However, in the peripheral blood, after careful searching of a sufficient quantity of blood ($\frac{1}{4}$ cubic millimetre), a few rounding or rounded parasites can generally be seen (figs. 12, 17-20). Also, in the internal organs, stages of the parasite intermediate between the flagellate and non-flagellate forms may be seen (fig. 33).

The post-flagellate or latent bodies vary in size (figs. 2-16, 22). This variation is due to two causes : (1) the formation of non-flagellate parasites from trypanosomes of different breadths, and (2) the occasional division of large post-flagellate forms by binary fission, an example of the simplest schizogony (fig. 23). This fission, so far as my researches go, is infrequent in the case of *T. gambiense* and *T. rhodesiense*. It has been observed that division of flagellate trypanosomes may immediately precede the formation of latent bodies.

Broad forms of *T. rhodesiense*, with posterior nucleus, may form relatively large latent bodies. As the nucleus is at or near the posterior (non-flagellar) end of the parasite, there is little of the body discarded in that region when rounding occurs. The kidney shape of the nucleus of some specimens of *T. rhodesiense* is seen in their latent bodies (fig. 27), and the nucleus lies to one side of the rounding body (figs. 17, 18). It is not easy, however, to differentiate between the latent bodies of *T. rhodesiense* and *T. gambiense*.

Moore and Breinl describe a stainable band or black line connecting the nucleus and blepharoplast of certain specimens of *T. gambiense* at or near the maxima. An 'interaction' takes place between the blepharoplast and nucleus. After this the formation of latent bodies proceeds. During the researches now recorded, there was no good evidence found in support of Moore and Breinl's views. The stainable band was seen in some stout (probably old) parasites, and in parasites at periods near the death of the host.

It is possible, as Swellengrebel (1908) suggested, that the stainable line is a form of degeneration. However, on the exact significance of the stainable band seen in such trypanosomes I prefer not to pronounce a definite opinion at present.

THE METAMORPHOSIS OF LATENT BODIES INTO TRYPANOSOMES

This process was observed in life on several occasions, though the complete passage from a rounded body to a fully flagellate moving trypanosome was only rarely seen (three times), for it is difficult to imitate precisely the natural conditions favourable to such a metamorphosis. However, by taking rounded bodies, usually obtained from the spleen of an infected rat, in a little physiological salt solution, and adding thereto an equal quantity of fresh (normal, uninfected) rat's blood, some of the rounded bodies were seen on a warm stage (25° to 35° C.) to grow, each becoming larger and sending out a process (text-fig. 2). This pseudopodium-like process lengthens, and a flagellum is formed from an area close to

TEXT-FIG. 2 represents diagrammatically the metamorphosis of a rounded, latent or non-flagellate parasite into a flagellate trypanosome (*T. gambiense*). The rounded bodies, obtained from the spleen of an infected rat, were placed in warm, fresh, uninfected rat's blood and watched under the microscope. The total time taken for the metamorphosis was about one hour.

the blepharoplast (kinetonucleus). At this stage the parasite forms at its drawn-out anterior end an undulating membrane along the edge of which the flagellum lies, and the organism somewhat resembles a *Crithidia*. This transitory stage may be termed the crithidial stage. The organism grows and the blepharoplast passes posterior to the nucleus, and the trypaniform stage is assumed.

The evidence of stained preparations (figs. 32 to 40) fully supports this mode of formation of flagellate trypanosomes from non-flagellate latent bodies. The latent bodies, which are the post-flagellate stages of one generation of trypanosomes, become the pre-flagellate stages of the succeeding generation of trypanosomes. Such pre-flagellate, Crithidia-like parasites in various stages of metamorphosis may be seen in the peripheral blood of the host (figs. 32, 34 to 42), when the parasites are increasing in numbers therein.

A basal granule (blepharoplast of Minchin) is seen at the base of the flagellum of some stained flagellating parasites (fig. 37).

It may be added that in dealing with an isolated case of an intermediate stage of a parasite between the flagellate and the rounded body, it is sometimes difficult to determine whether the given stage is pre-flagellate or post-flagellate, that is, whether the given parasite is proceeding in development towards the flagellate stage or away from it towards the rounded body.

THE SIGNIFICANCE OF THE NON-FLAGELLATE OR LATENT FORMS OF TRYPANOSOMES. INOCULATION WITH LATENT BODIES PRODUCES TRYPANOSOMIASIS

R. Ross and D. Thomson (1910) report periodic variations in the numbers of the trypanosomes found in the blood of a patient, W. A., suffering from Rhodesian Sleeping Sickness. Fantham and J. G. Thomson (1910) report similar periodic variation in the number of the parasites in the peripheral blood of sub-inoculated animals (rats, guinea-pigs, and rabbits). During the periods of decrease of the parasites in the peripheral blood, I find that latent (non-flagellate) bodies are present in relatively large numbers in the internal organs of the host. The latent bodies are formed at or near the period of maximum increase of the trypanosomes in the peripheral blood. The latent bodies are especially numerous in

the spleen and bone marrow on the downward slope of the curve representing the numbers of the parasites in the peripheral blood of the host. Change of the latent forms into trypanosomes takes place on the rise or upward slope of the curve.

There is, of course, a mutual action and reaction of the host and the parasite, the resistance of the host probably being greatest when the flagellate trypanosomes within it are beginning to decrease, thus helping to bring about the assumption of the rounded form by many of the flagellates, so that latent or resistant non-flagellate stages of the parasite are then numerous.

The occurrence of latent bodies also helps to explain the successful inoculation of animals with trypanosomiasis when no flagellates can be found in the blood inoculated from a previously infected animal. Although it might be urged that flagellate trypanosomes in numbers too few to recognise may actually be present in the infected blood inoculated, yet it is possible to inoculate only latent, non-flagellate bodies, and give the inoculated animal trypanosomiasis. In other words, persistent infectivity in the case of trypanosomes is explained by rounded bodies.

I have performed this experiment (inoculation of latent bodies) on two occasions. In the first experiment a rat was inoculated with one drop of spleen-pulp (from Rat 7) mixed with a little sterile physiological salt solution, the mixture containing no flagellates. The inoculated rat developed trypanosomiasis on the 6th day, dying on the 12th day. The daily counts of this rat were as follows:—

Day	1	2	3	4	5	6	7
Parasites per c.mm.	—	—	—	—	—	4	864

Day	8	9	10	11	12
Parasites per c.mm.	2196	4308	30,600	10,000	75,200

Secondly, a further experiment was tried, since it was considered that the parasites in one drop of spleen-pulp solution might be too numerous to count accurately, and that a few flagellate

trypanosomes might be contained therein, for although a most minute search was made for flagellates in the fluid before inoculation, yet a very few flagellates might have remained undetected. Accordingly, in the second experiment, 1 cubic millimetre of spleen-pulp solution in sodium citrate was inoculated intraperitoneally. Careful examination of samples of the solution used, both fresh and stained, showed no flagellate trypanosomes, but many rounded bodies. The actual fluid used for inoculation also showed no flagellate trypanosomes when microscopically examined fresh. The rat thus inoculated developed trypanosomiasis, parasites being found on the 8th day, and it died on the 18th day. The daily counts of this rat are appended:—

Day	1	2	3	4	5	6	7	8	9	10	11
Parasites per c.mm.	—	—	—	—	—	—	—	4	960	2800	12,000

Day	12	13	14	15	16	17	18
Parasites per c.mm.	49,200	18,200	6400	2120	20,600	50,240	128,000

The two rats inoculated with non-flagellate, latent bodies of *T. rhodesiense* showed incubation periods rather longer (5 to 7 days) than usual (2 to 4 days) with rats inoculated with flagellate *T. rhodesiense*.

The occurrence of non-flagellate bodies in the life-cycle of the parasites of sleeping sickness also explains recurrence of trypanosomiasis after it has apparently died out in an infected animal. In such cases the latent bodies are present in the host all the while in such organs as the spleen and bone marrow.

During these researches it has been found that flagellate trypanosomes (500,000 to 2,000,000 in number) inoculated into a rat or a guinea-pig can be detected in the peripheral blood-stream (in 1 cubic millimetre of blood) of the host for some ten to twelve hours, or even eighteen hours after inoculation. During and after this period, that is, during the incubation period of the parasite,

a few rounded forms of trypanosomes can be found in a cubic millimetre of peripheral blood.

Biot (1910) writes of the 'revivifying action' of physiological salt solution on trypanosomes (*T. lewisi*), especially in fluid from the liver of a rat dead six and a half days (kept in the cold, unopened). Biot does not explain the phenomenon. However, it is capable of explanation, for in the liver latent bodies of trypanosomes are present, which, under the relatively favourable environment of isotonic salt solution, flagellate and become typical trypanosomes.

In the treatment of trypanosomiasis by drugs, careful note must be taken of the occurrence of rounded, non-flagellate or latent forms of the parasite. A drug needs to be found which will either prevent the formation of rounded (latent) stages or disintegrate those latent bodies already formed. In this connection the work of B. Moore, Nierenstein, and Todd* on the combined use of salts of mercury and arsenic should be considered.

NOTE ON THE DEGENERATION OF TRYPANOSOMES

All flagellate trypanosomes do not become rounded and form latent, non-flagellate bodies, but some of them degenerate and die. The degeneration may take various forms—some become (α) somewhat irregular and almost amoeboid with pale-staining cytoplasm and vacuoles (figs. 44, 45), others (β) exhibit chromatolysis, wherein the nucleus becomes poor in chromatin and chromatoid granules occur in the cytoplasm (figs. 46-49), while others (γ) exhibit marked vacuolation (fig. 50). Such degenerating forms may be seen in various internal organs of the host, such as the lungs and spleen, especially during the period of formation of latent bodies.

It is also very probable that some of the latent bodies themselves die and do not flagellate, for some shrunken latent bodies, with undifferentiated contents, can be seen in the spleen.

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* Ann. Trop. Med. and Parasitol. (1907), Vol. I, pp. 275-284.

SUMMARY AND CONCLUSIONS

1. Non-flagellate stages of trypanosomes, such as *T. gambiense* (Dutton) and *T. rhodesiense* (Stephens and Fantham), occur.

2. These non-flagellate stages ('latent bodies' of Moore and Breinl) are especially found in the lungs, spleen and bone marrow, during periods of decrease of trypanosomes in the peripheral blood of the host.

3. They are in process of formation at or near the time when the trypanosomes are most numerous in the peripheral blood. The formation of latent bodies takes place especially in the lungs, and they collect in the spleen and bone marrow of the host.

4. In the formation of non-flagellate stages, some of the cytoplasm and the flagellum of the trypanosome are disintegrated. The non-flagellate body contains the nucleus and blepharoplast (kinetonucleus) of the trypanosome.

5. Non-flagellate (latent) bodies can be seen to grow and flagellate, turning into trypanosomes, when placed in fresh, warm, uninfected blood.

6. Latent bodies of *T. rhodesiense*, inoculated into a rat, flagellate and produce trypanosomiasis.

7. The non-flagellate (latent) bodies of trypanosomes (*T. gambiense* and *T. rhodesiense*) are the post-flagellate stages of one generation of trypanosomes and the pre-flagellate stages of the succeeding generation of trypanosomes.

8. There is a life-cycle of trypanosomes (*T. gambiense* and *T. rhodesiense*) in Vertebrate hosts, comparable with those of *Crithidia* and *Herpetomonas* in the alimentary tracts of various Invertebrates. The latent (relatively resistant) stages of trypanosomes occurring in Vertebrates are separate from, and in addition to, stages of the parasite which may occur in the Invertebrate carrier (for example, *Glossina*).

9. In the treatment of trypanosomiasis by drugs, careful note must be taken of the occurrence of rounded non-flagellate or latent forms of the parasite. A drug needs to be found which will either prevent the formation of rounded (latent) stages, or disintegrate those latent bodies already formed.

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EXPLANATION OF PLATE XXVII.

All figures were outlined with Abbé-Zeiss camera lucida, using 2 mm. apochromatic homogeneous immersion objective and compensating oculars 8 and 12 of Zeiss. Magnification 2,000 diameters approximately, except where otherwise stated.

The chief stains used were iron haematoxylin, Giemsa, or Romanowsky after wet fixation.

The figures represent *T. rhodesiense*, as seen in rats, except where otherwise indicated.

Fig. 1 represents the formation of latent (non-flagellate) body from trypanosome in heart blood.

Figs. 2-5 represents latent bodies, with remains of trypanosomes around or near, from heart (fig. 4) and lungs.

Figs. 6, 7.—Oval latent bodies, each with nucleus and blepharoplast (kinetonucleus), from lung of rat and peripheral blood of guinea-pig respectively.

Figs. 8-16.—Various forms of latent bodies—from liver, heart, spleen, lung (figs. 8-11); of *T. gambiense* in peripheral blood of rat (fig. 12); from peripheral blood (fig. 13), lung, and heart respectively.

Figs. 17-20.—Rounding, herpetomonad-like forms, all from peripheral blood of guinea-pigs. Fig. 19 of *T. gambiense*.

Fig. 21.—Broad trypaniform parasite from blood of guinea-pig.

Fig. 22.—Latent form of *T. gambiense* from lung of rat.

Figs. 23-24.—Possible division forms of latent bodies of *T. rhodesiense* from heart and lungs of rats. Fig. 24 magnified 1,350 diameters. These forms are rare.

Figs. 25, 26.—Pyriform latent bodies, each with nucleus and blepharoplast. Fig. 25, of *T. rhodesiense* from blood of guinea-pig; fig. 26, of *T. gambiense* from blood of rat.

H. B. Fantham, del.

Huth, Lith^r London

LATENT & OTHER FORMS OF
TRYPANOSOMA GAMBIENSE & T. RHODESIENSE.

Figs. 27, 28.—Pyriform bodies of *T. rhodesiense* and *T. gambiense* (fig. 28) from peripheral blood of guinea-pig. In fig. 28 the flagellum is shown beginning to develop.

Figs. 29, 30.—Two pyriform non-flagellate forms from spleen of rat.

Fig. 31.—Round form of *T. gambiense*, with nucleus, blepharoplast and short flagellum along the edge of the body; from peripheral blood of rat.

Figs. 32-40.—Crithidia-like forms. Fig. 32, of *T. gambiense* from blood of rat; figs. 33-35, of *T. rhodesiense* from lung (fig. 33) and peripheral blood of rat; figs. 36-38, of *T. gambiense* from blood of guinea-pigs; figs. 39-40, of *T. rhodesiense* from blood of guinea-pigs. In fig. 37 note the basal granule ('blepharoplast' of Minchin) at the root of the flagellum, forming the centrosome of the kinetonucleus.

Fig. 41 represents an almost trypaniform stage of *T. gambiense* from blood of guinea-pig. Note the peculiar position of the blepharoplast (kinetonucleus).

Fig. 42 represents a stout trypaniform parasite from heart blood of rat.

Fig. 43 shows peculiar, large rounding form (from blood of a rat), exhibiting signs of division of its nucleus and blepharoplast.

Figs. 44, 45.—Slightly irregular parasites, very pale staining and vacuolated. Degenerating, as seen in detachment of flagellum, etc.

Figs. 46-49.—Degenerating trypanosomes, exhibiting chromatolysis, all from lungs of rats. Fig. 46 of *T. gambiense*, magnified 1,350.

Fig. 50.—Degenerating *Trypanosoma gambiense*, showing vacuolation, pale-staining cytoplasm and beginning of disintegration.

EXPERIMENTS ON THE TREATMENT OF ANIMALS INFECTED WITH TRYPANOSOMES BY MEANS OF ATOXYL, VACCINES, COLD, X-RAYS AND LEUCOCYTIC EXTRACT; ENUMERATIVE METHODS EMPLOYED*

BY

PROF. MAJOR R. ROSS, C.B., F.R.S.,

AND

J. G. THOMSON, M.A., M.B., CH.B.

(*Received for publication* 15 *October*, 1910)

These experiments were conducted with funds given by Sir Edwin Durning-Lawrence, Bart., for the purpose of testing the effect of cold on disease, and were suggested by one of us (R.R.) as a part of the studies made in connection with his case of sleeping sickness, as reported upon in an accompanying paper by him and Dr. David Thomson. Although many researches have been made on the effect of atoxyl and other drugs, we believe that these are the first in which that effect has been measured by regular daily counts of the parasites by measured thick-film methods.

I. ATOXYL

(*a*) *Small doses.*

From a study of the patient, W.A., at the Royal Southern Hospital by Major R. Ross and D. Thomson it will be noted that atoxyl failed to be of any marked benefit to the patient, and in the doses administered there was no trypanocidal action detected. It was necessary, therefore, to begin a series of experiments on animals inoculated with *T. rhodesiense* (Stephens and Fantham †),

* An abstract of this paper was read before the Royal Society on December 8th, 1910, and published in Proc. Roy. Soc., B, Vol. LXXXIII, pp. 227-234 (1911).

† Proc. Roy. Soc., Series B, Vol. LXXXIII, pp. 28-33 (1910), and Ann. Trop. Med. and Parasit., Vol. IV, pp. 343-351.

and to try the effect of small doses of atoxyl in these animals with a view to determine the effect of that drug on this particular trypanosome which, as will be seen from the accompanying paper on 'Enumerative Methods in Untreated Animals' (H. B. Fantham and J. G. Thomson*), was of extraordinary virulence. Various doses of atoxyl are recommended in treatment of human trypanosomiasis, and we find that the patient, W.A., in the Royal Southern Hospital, received four grains as a maximum dose. It was impossible in the case of this patient to push the drug further as he quickly showed signs of the toxic action of the drug. Louis Martin and Henri Darré†, in an article on the treatment of sleeping sickness at the Pasteur Hospital, believe that atoxyl can cure light forms of the disease, and even severe forms, but that it is often necessary to give large doses, viz., one gram, which imperil vision. These authors however, still admit that permanent cure is a doubtful matter.

In the experiments made by us we attempted, as far as possible, to administer a therapeutic dose of atoxyl. We first sub-inoculated four rats subcutaneously with *T. rhodesiense*. The lightest in weight, Rat 34, weighed 139 grams, and the heaviest weighed 256 grams, namely, Rat 37.

	Colour	Weight	Inoculation period	Duration of life	
Rat 34 ...	Piebald	139 grams	5 days	27 days	Animal House
Rat 35 ...	Piebald	145 grams	5 days	26 days	Cold Chamber
Rat 36 ...	White	251 grams	6 days	27 days	Cold Chamber
Rat 37 ...	White	256 grams	5 days	8 days	Animal House

All the four rats were adults. Two were piebald and two white, and all received, subcutaneously, the same dose of trypanosomes, namely, 200,000. Two of them, 35 and 36, were placed in the cold chamber, where the lowest temperature recorded was 20° F., and two were placed in the animal house. All these animals received atoxyl in solution subcutaneously in small repeated doses, which varied from $\frac{1}{60}$ to $\frac{1}{20}$ of a grain. Take the body weight of a man as being

* Proc. Roy. Soc., B. Vol. LXXXIII, 1911, pp. 206-211, and Ann. Trop. Med. and Parasit., Vol. IV, pp. 417-463.

† Bulletin of Sleeping Sickness, Vol. II, No. 18, 1910.

70 kilograms and the weight of our four rats as varying between 139 grams and 256 grams, and we find that the doses we administered to the rats, when calculated to the body weight of a man would represent doses varying between 4·55 grains and 25·14 grains. Rats 34 and 35, being the lighter rats, having received the same doses as Rats 36 and 37, therefore received a relatively larger dose when we consider the body weight. We now append tables of numbers of trypanosomes per c.mm. of peripheral blood and the doses of atoxyl given to Rats 34, 35, 36 and 37, and we also give graphs of the Rats 34, 35 and 36, which show the variations of the numbers of parasites in the peripheral blood.

RAT 34.—Piebald, weight 139 grams. Treated in Animal House with small doses of atoxyl. Inoculation dose: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood	—	—	—	—	—	1,392	26,960
Atoxyl in grains	—	—	—	—	—	—	$\frac{1}{60}$

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	40,460	8,118	1,440	10,500	5,888	101,200	96,800
Atoxyl in grains	$\frac{1}{60}$	—	$\frac{1}{60}$	—	—	$\frac{1}{60}$	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood	73,920	4,000	20,496	5,796	12,696	3,864	19,136
Atoxyl in grains	$\frac{1}{30}$	—	$\frac{1}{30}$	—	$\frac{1}{20}$	—	$\frac{1}{30}$

Day	22	23	24	25	26	27	—
Number of Trypanosomes per c.mm. of blood	24,000	73,920	48,000	42,320	168,960	242,880	—
Atoxyl in grains	—	$\frac{1}{30}$	$\frac{1}{30}$	—	—	$\frac{1}{30}$	—

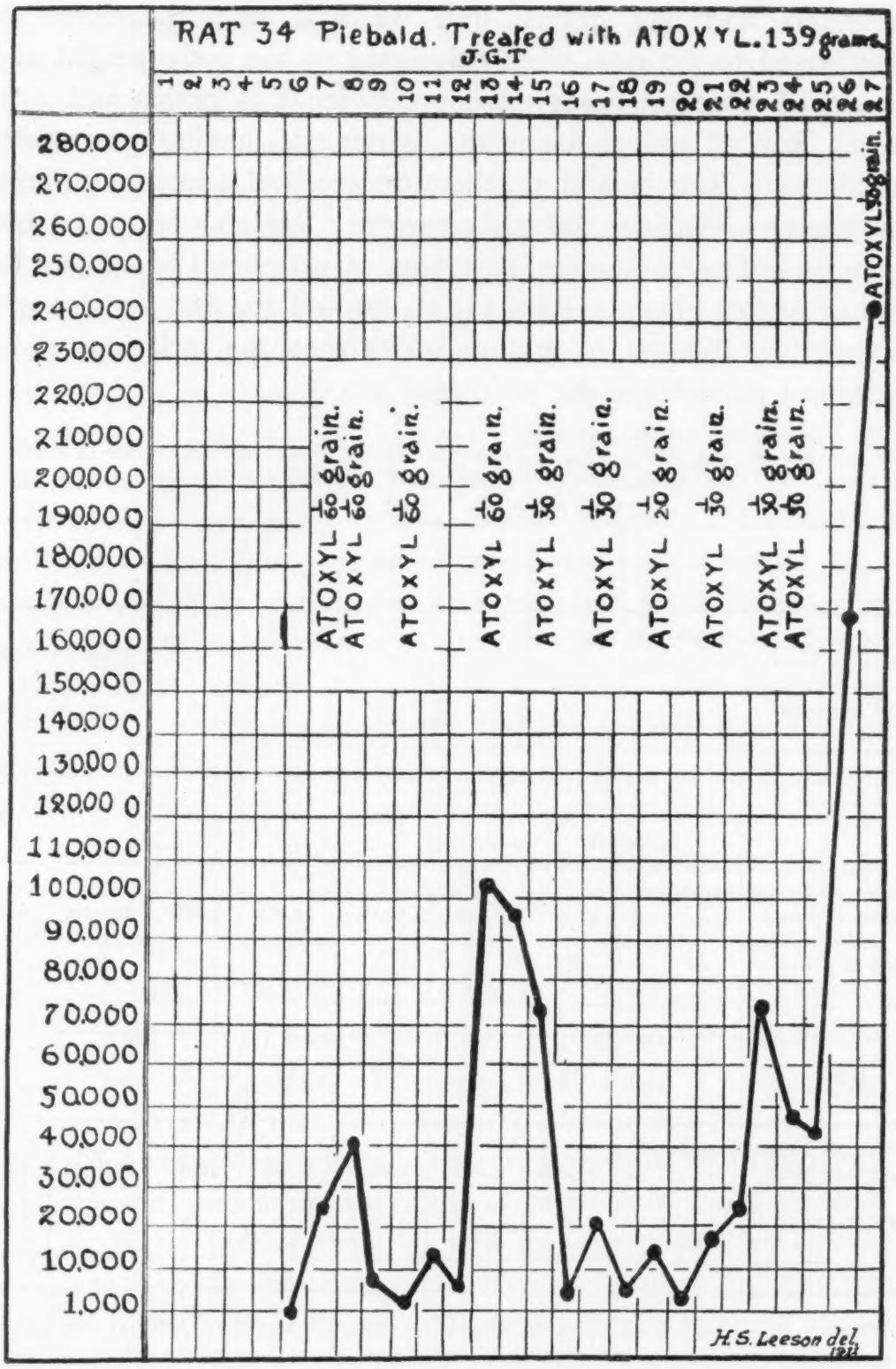

CHART 1.—Rat 34, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Count of parasites made every 24 hours per c.mm. of peripheral blood.

RAT 35.—Piebald Adult Rat, weight 145 grams. Treated in Cold Chamber with small doses of atoxyl. Dose of inoculation: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood	—	—	—	—	—	2,064	25,112
Atoxyl in grains	—	—	—	—	—	—	$\frac{1}{60}$

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	10,400	27,968	34,320	1,520	72,128	73,920	154,000
Atoxyl in grains	$\frac{1}{60}$	—	$\frac{1}{60}$	—	—	$\frac{1}{60}$	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood	80,560	230,400	221,600	18,800	331,296	384,384	338,688
Atoxyl in grains	$\frac{1}{30}$	—	$\frac{1}{30}$	—	$\frac{1}{30}$	—	$\frac{1}{30}$

Day	22	23	24	25	26	—	—
Number of Trypanosomes per c.mm. of blood	390,000	550,000	447,680	383,040	570,000	—	—
Atoxyl in grains	—	$\frac{1}{30}$	$\frac{1}{30}$	—	—	—	—

RAT 36.—White Adult, weight 251 grams. Treated in Cold Chamber with small doses of atoxyl. Dose of inoculation: 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm. of blood	—	—	—	—	—	—	192
Atoxyl in grains	—	—	—	—	—	—	—

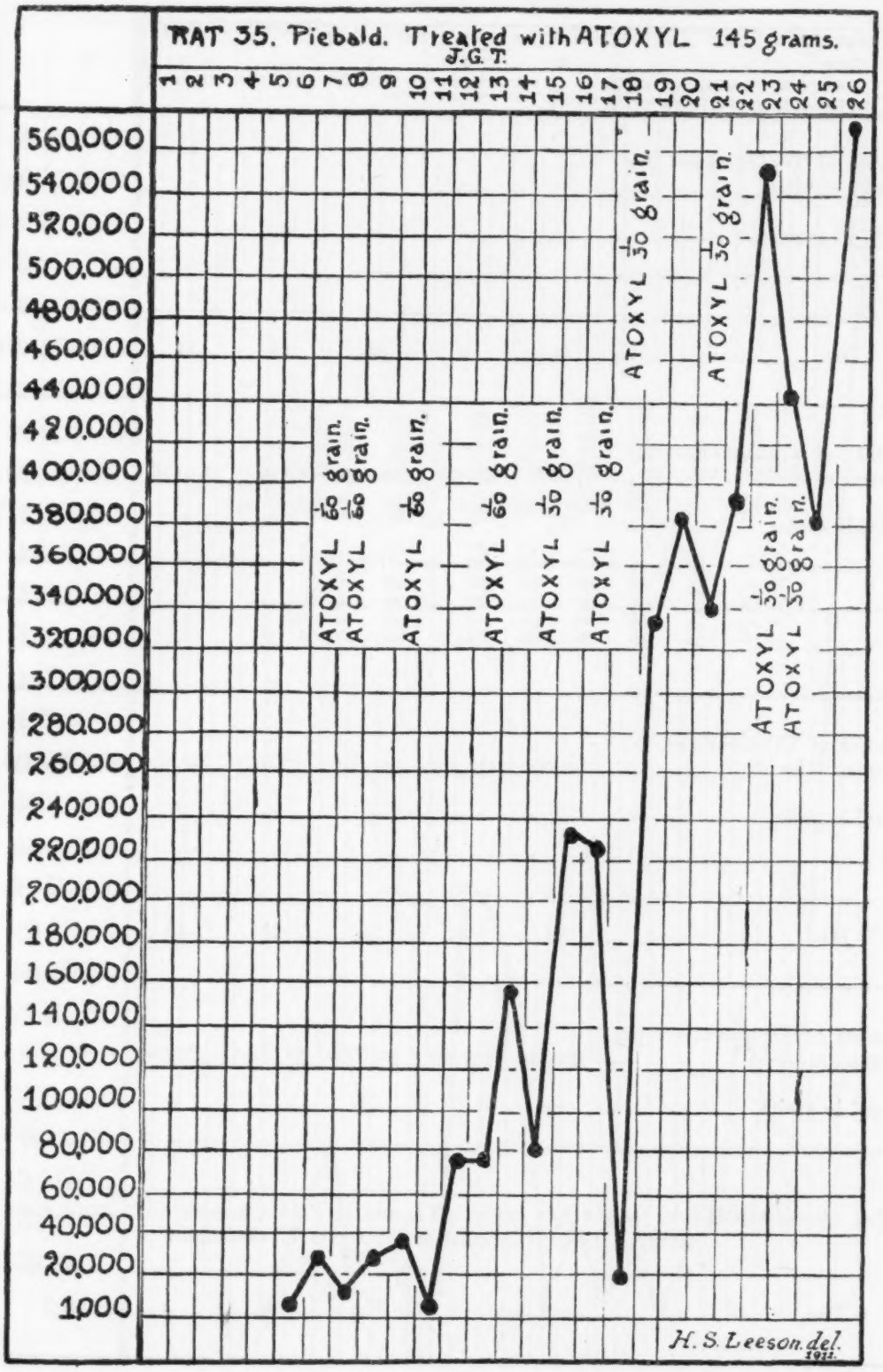

CHART 3.—Rat 35, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Count of parasites made every 24 hours per c.mm. of peripheral blood.

Rat 36—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm. of blood	34,356	70,560	75,600	80,640	168,544	66,528	125,400
Atoxyl in grains	$\frac{1}{60}$	$\frac{1}{60}$	—	—	—	$\frac{1}{60}$	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm. of blood	300,000	241,000	336,000	316,800	404,800	337,920	363,000
Atoxyl in grains	$\frac{1}{30}$	—	—	—	$\frac{1}{20}$	—	$\frac{1}{30}$

Day	22	23	24	25	26	27	—
Number of Trypanosomes per c.mm. of blood	410,000	281,600	138,000	530,000	483,840	500,000	—
Atoxyl in grains	—	$\frac{1}{30}$	$\frac{1}{30}$	—	—	$\frac{1}{30}$	—

Rat 37.—White, weight 256 grams, Adult. Treated in Animal House with small doses of atoxyl. Animal developed Pneumonia

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm. of blood	—	—	—	—	—	4	2,004	32,640
Atoxyl in grains	—	—	—	—	—	—	$\frac{1}{60}$	$\frac{1}{60}$

If now we examine table and chart of Rat 34 we find that the period between the crests of the waves representing the numbers of trypanosomes was certainly longer than in those of the untreated animals inoculated with *T. rhodesiense* (*vide* Paper on 'Enumerative Methods in Untreated Animals,' H. B. Fantham and J. G. Thomson*). We find there were two high crests in the graph of this animal corresponding to five days and ten days. The weight of this animal was 139 grams, and thus the doses administered were

* Proc. Roy. Soc., B, Vol. LXXXIII, 1911, pp. 206-211, and Ann. Trop. Med. and Parasit., Vol. IV, pp. 417-463.

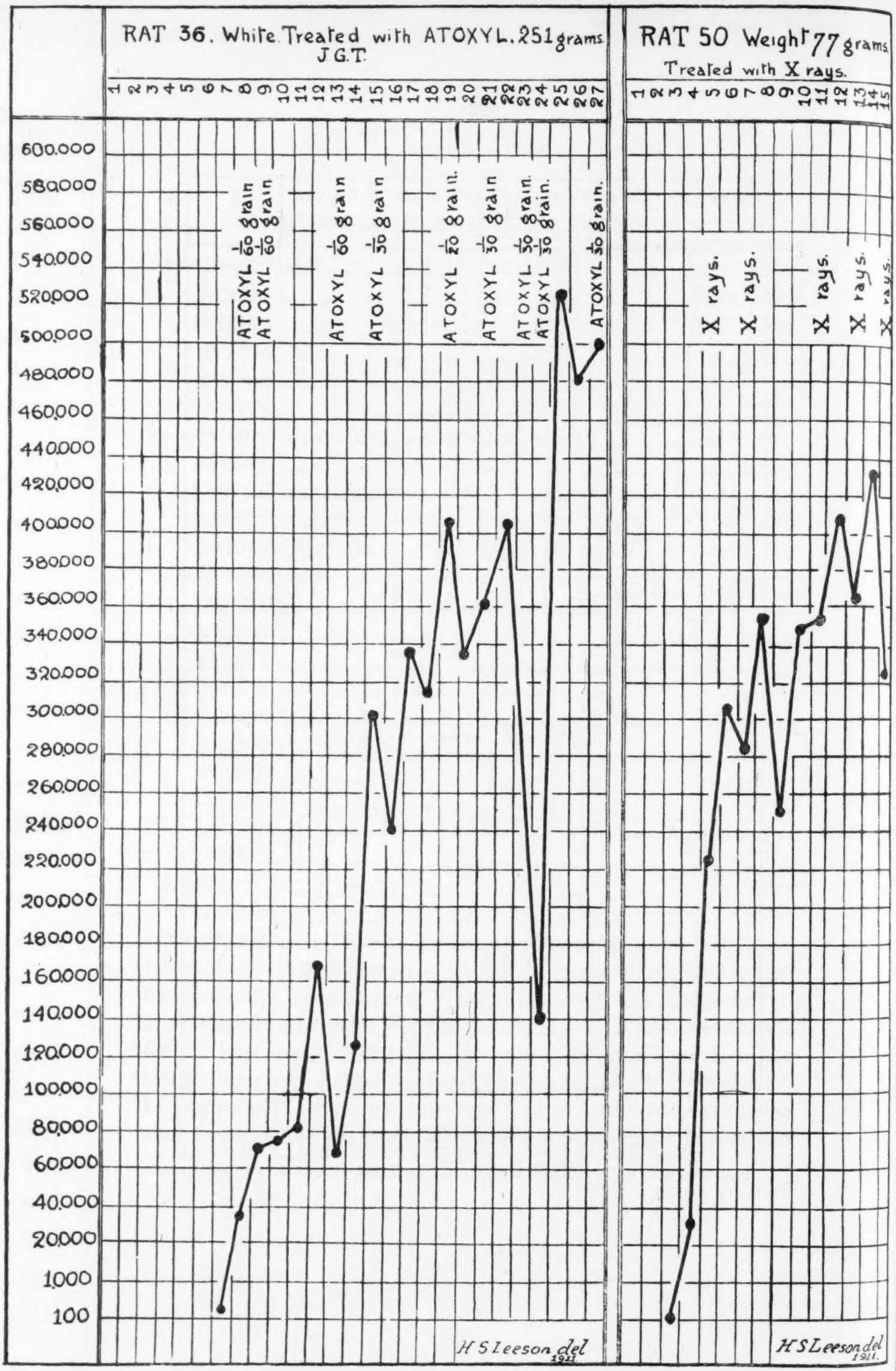

CHART 3.—Rats 36 and 50, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Counts made every 24 hours per c.mm. of peripheral blood.

relatively large. The doses given may have had a slight trypanocidal action, but from the appearances of the smears we do not think there was any marked degeneration, and many trypanosomes were found actively dividing. The doses given to this animal would, if calculated to the body weight of a man of 70 kilograms, represent approximately 8·38 grains, 16·76 grains, and 25·14 grains. The lengthened period between the crests of the waves may have been caused by natural body resistance being raised. This animal lived twenty-seven days, whereas the longest period of life recorded in our controls, sub-inoculated with the same strain, was eighteen days in an animal weighing 48 grams.

If we examine now Rat 35, which was treated with similar doses of atoxyl, and kept in the cold chamber, we find quite a different character in the graph representing the numbers of trypanosomes in the peripheral blood. This animal was piebald in colour and weighed 145 grams, that is to say, it was only slightly heavier than Rat 34. This means that the doses given when calculated to the body weight of a man were smaller than those given to Rat 34. Here the doses would represent in man 8·04 grains, 16·08 grains and 24·12 grains. In spite of these doses the trypanosomes in the peripheral circulation rapidly increased in numbers with a staircase effect, and on the day before death reached the enormous number of 570,000 per c.mm. of blood. This animal lived twenty-six days, and received during that time ten doses of atoxyl. It cannot be said in this case that the atoxyl in the doses given had any trypanocidal action, but, again, the life of the animal was undoubtedly prolonged by the drug. The numbers of division forms seen in the smears of this animal showed that the proliferation of the trypanosomes was very active, and we also noted that the proportion of short, stumpy forms was also markedly increased.

Rat 36, white in colour, and treated in the cold chamber with small doses of atoxyl, shows a chart which in many respects simulates the chart of Rat 35. Here, again, we note the staircase rise of the trypanosomes in spite of continued treatment with small doses of atoxyl. This animal being heavier than Rats 34 and 35, received, therefore, doses which, when calculated to the body weight of a man, were relatively small, namely, 4·64 grains, 9·28 grains, and 13·92 grains.

In this animal all the smears gave evidence of the trypanosomes being abnormally stimulated to division. Many of the trypanosomes were seen to be dividing into four, and the numbers before death reached 530,000 per c.mm. of blood.

This animal lived twenty-seven days. If we do not attribute the prolonged life of these three rats to the trypanocidal action of the atoxyl we must endeavour to explain it in another manner.

Arsenic in small doses affects metabolism and improves the general condition of the body. In small doses we find in man, both in health and disease, that the strength, weight and appetite are improved, and it is quite to be expected, therefore, that small repeated doses of atoxyl in rats would tend to raise the natural body resistance. In referring to Cushny's *Pharmacology* we find that Gies treated young rabbits with arsenic, and found that those treated weighed more, had larger bones, and more developed muscles than the untreated controls. It was also found that a pregnant female rabbit treated with arsenic gave birth to young rabbits of abnormal size. That arsenic, therefore, in small doses profoundly affects metabolism and improves the general condition we have good evidence, and this might possibly explain the lengthened life of the animals treated with atoxyl in small doses. In the same way the lengthened period between the crests of the waves in Rat 34 may possibly have been due to increased resistance of the body of the host rather than to a direct action of the drug administered upon the parasites.

In spite of the fact that the animals' lives were prolonged we had the trypanosomes increase steadily in numbers, and this was very marked in the cases of Rats 35 and 36. The numbers of trypanosomes in the peripheral blood remained consistently high, and the smears showed very active divisions of parasites. This can be attributed to the action of the atoxyl in small doses. We have already referred to the general action of arsenic in small therapeutic doses. The drug seems to act as a tonic to the body in health as well as disease, and must, therefore, improve the condition of the cells of the whole body. Stockman and Greig* found the bone marrow in a state of unusual activity when arsenic was administered

* See Cushny's 'Pharmacology' on actions of arsenic.

in small doses, and there were greater numbers of red blood corpuscles.

Is it not possible, therefore, that atoxyl in small doses, such as given to these rats acted not only upon the cells of the body of the host, but on the trypanosomes themselves, and acted as a tonic to both, so stimulating increased division of the parasites? This would explain both the extraordinary division forms of the trypanosomes and also the prolonged life of the host without attributing to the atoxyl any trypanocidal action whatever.

It must be remembered, however, that we are working with a specially virulent strain of trypanosomes, and that, therefore, it may be peculiarly resistant to the action of atoxyl, which drug in many cases of human sleeping sickness seems undoubtedly to have a favourable influence on the disease.

Rats 35 and 36 were treated in the cold chamber and received atoxyl as well. In our experience, however, we find that although the cold tends to prolong the life of animals infected with trypanosomiasis to a slight extent it has not, as a rule, any marked effect upon the numbers of trypanosomes in the peripheral circulation, and thus we attribute the numerous division forms to the atoxyl.

(*b*) *Large doses of Atoxyl.*

We now tried the effect of a large dose of atoxyl on three rats, all heavily infected with *T. rhodesiense*.

Here we were guided in our experiments by the work of Thomas and Breinl*, who pointed out that if a cure was to be expected in animals a large dose of atoxyl was necessary.

Rat 38.—Piebald, weight 202 grams. (See Chart.) Dose of inoculation: 200,000 Trypanosomes. *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	202	6,162
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

* Memoir XVI, Trypanosomiasis research, pp. 52-57, Sch. of Trop. Med., Liverpool, October, 1905.

Rat 38—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	28,000	86,000	43,000	21,736	0	0	0
Atoxyl 5 per cent. solution ...	—	—	—	0·5 c.c.	—	—	—
Leucocytes	—	—	—	16,368	24,720	63,000	40,000

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	0	1,600	18,000	32,000	120,000	98,000	89
Atoxyl 5 per cent. solution ...	—	—	—	—	—	0·5 c.c.	—
Leucocytes	22,880	—	—	—	—	12,360	24,000

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	320	4,510	12,000	26,000	60,000	23,600	20,310
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	45,000	—	—	—	—	—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	7,500	760	84	320	3,600	4,880	15,600
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	23,000	79,488	123,600	193,000	177,744	350,000	144,000
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	—	33,120	16,000	—	40,320	10,400	4,248

Rat 38—continued.

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	317,856	218,880	231,840	211,520	112,640	190,520	664,608
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	21,924	—	31,020	9,280	26,048	19,404	33,120

Day	50	51°	—	—	—	—	—
Number of Trypanosomes per c.mm.	816,960	1,500,000	—	—	—	—	—
Atoxyl 5 per cent. solution ...	—	—	—	—	—	—	—
Leucocytes	63,480	—	—	—	—	—	—

The above animal, Rat 38, lived fifty-one days. The control of this animal weighing 238 grams lived eighteen days, and the day before death had 350,000 trypanosomes per c.mm. Rat 38 received two doses of a 5 per cent. solution of atoxyl (0·5 c.c.) subcutaneously, and it will be noted that this dose calculated to the body weight of a man of 70 kilograms would be 130 grains approximately. It is of interest to note that the leucocytes rose in Rat 38 on the thirteenth day to 63,000 per c.mm.

Two other rats infected with the same strain received doses of 0·5 c.c. of a 5 per cent. solution of atoxyl.

Rat 40, white, weight = 129 grams.
Rat 41, white, weight = 113 grams.

Rat 40 had 4,800 trypanosomes in the cubic millimetre before injection, and next day we found 89 per c.mm., i.e., twenty-four hours after injection of atoxyl, and in forty-eight hours there were no trypanosomes in the peripheral circulation.

Rat 41 had 7,056 trypanosomes per c.mm. before injection of atoxyl and next day two, presumably dead trypanosomes, were found in a thick film. This rat, however, succumbed to the toxic action of the drug the following day.

Atoxyl, therefore, in large doses is a trypanocide. Arsenic in so-called poisonous doses causes adverse conditions in cells, and

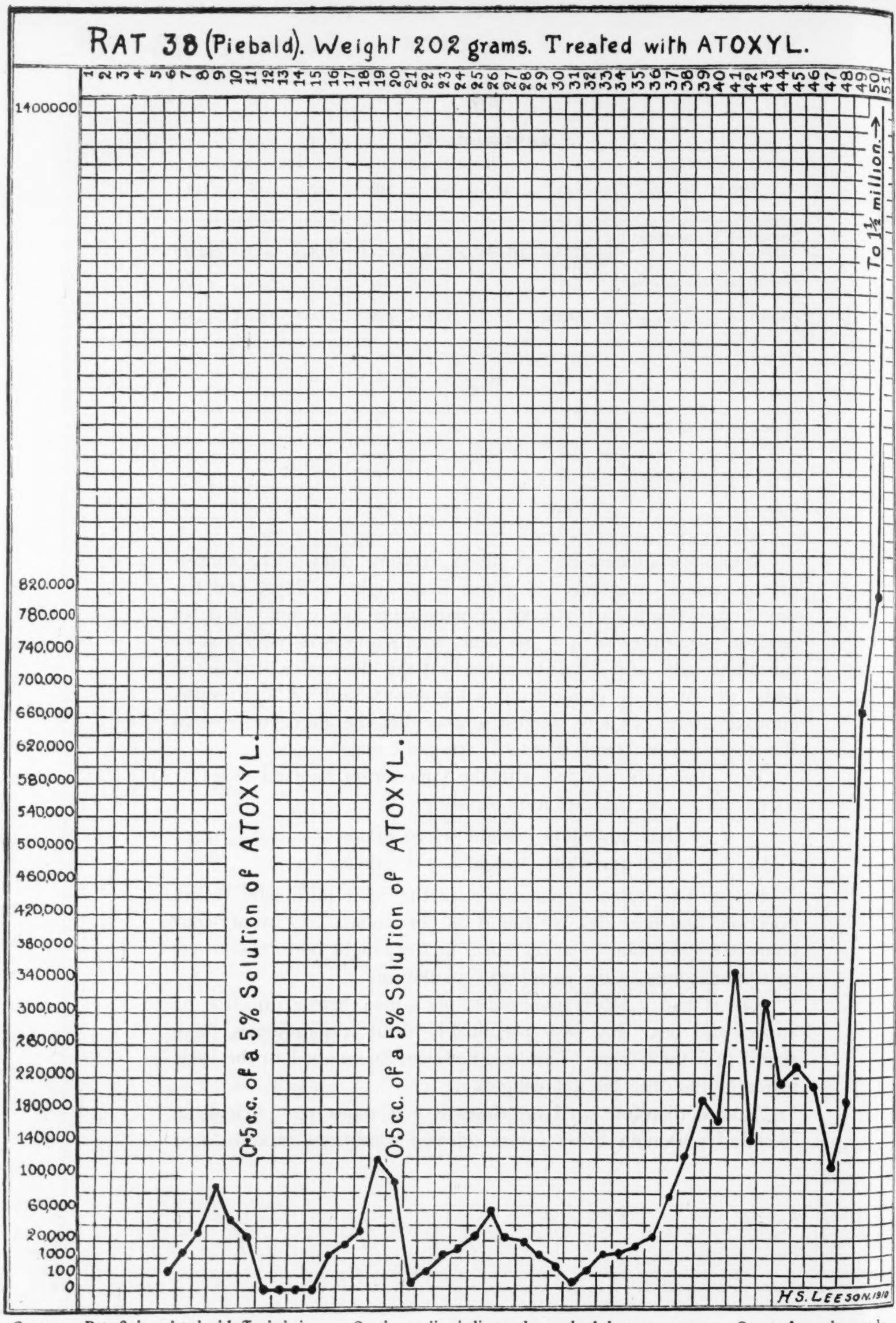

CHART 4.—Rat 38, inoculated with *T. rhodesiense*. Continuous line indicates the graph of the trypanosomes. Count of parasites made every 24 hours per c.mm. of peripheral blood.

we get fatty degeneration, especially of the cells of the liver and the epithelial cells of the stomach and intestines, and to a lesser extent in the kidneys and other organs in the body. If, therefore, we give atoxyl in a poisonous dose to an animal we kill not only the trypanosome cells, but also the body cells.

Unfortunately, in this strain of trypanosomes, even after the large dose of atoxyl given, the parasites appeared again in Rat 38 after an absence from the peripheral blood of four days. This is only to be explained by the resistant forms of Moore and Breinl (Ann. Trop. Med., I, page 457 (1907)) forming in the spleen and bone marrow, and these again flagellate out under favourable circumstances.

Thomas and Breinl pointed out that to obtain a cure we must approach as near as possible to the lethal dose.

A small dose of atoxyl repeated did not act as a trypanocide, but we would suggest prolonged the life of the animal by acting as a tonic.

A large dose of atoxyl, on the other hand, is certainly a trypanocide, and clears the peripheral circulation of the parasites, but in this strain, *T. rhodesiense*, which is a particularly virulent one, the trypanosomes were evidently able to resist the action of the atoxyl by encysting themselves in the spleen and bone marrow (Moore and Breinl*).

OUR CONCLUSIONS ARE BRIEFLY THESE:—

(1) Small doses of atoxyl, i.e., therapeutic doses in this strain, viz., *T. rhodesiense*, prolong the life of the host, probably by raising the body resistance.

(2) Small doses of atoxyl in this strain of trypanosomes actually stimulate division of the parasites.

(3) Large doses of atoxyl kill the parasites, but probably do not cure the animal owing to atoxyl-resistant bodies (Moore and Breinl) being formed in the spleen and bone marrow.

(4) As has been noted by other observers in virulent forms of this disease, the initial dose must be large if a cure is to be expected.

(5) Atoxyl is *not* a specific in *T. rhodesiense*, but would appear to be almost as toxic to the body cells as to the trypanosomes themselves.

* Ann. Trop. Med. and Parasit., Vol. I, p. 457 (1907).

Treatment of T. rhodesiense in rats by subcutaneous injection of vaccines.

II. VACCINE TREATMENT

In view of the extensive development of vaccine treatment in disease, we have carried out some experiments in the treatment of trypanosomiasis. These experiments are few, and therefore inconclusive, but they have produced results of interest, and we are continuing the experiments in the hope of gaining further knowledge.

The vaccine used by us was not a true vaccine, because it contained serum, blood corpuscles, leucocytes, and the bodies of dead trypanosomes. It will thus be noted that we injected a rather complicated fluid, and that it was only a vaccine in the sense that it contained the bodies of dead trypanosomes.

The vaccine was prepared by searing the surface of the heart with a hot needle in order to get a sterile portion, and through this the blood was drawn into a sterile pipette and mixed with normal saline. This mixture was placed for half an hour in an incubator at 55° C., and finally a little trikresol was added. A count was made of the parasites in 1 c.mm. before they were subjected to heat, and appropriate dilution was carried out with normal sterile salt solutions.

There are two distinct points at which we may prepare this *so-called* vaccine. We may prepare it at the height of the rise of the trypanosomes in the peripheral blood, or it may be prepared when the trypanosomes are few in number.

In the former case we have a condition in which the numbers of trypanosomes are great, and probably the anti-bodies in the serum few. In the latter case we have, on the other hand, a condition where the trypanosomes are few and the serum rich in anti-bodies.

We have conducted experiments and used a so-called vaccine prepared at both the above stages. The first so-called vaccine was rich in trypanosomes, and was, therefore, more of the nature of a true vaccine than the latter, which was what we might call a vaccine *plus* an anti-serum.

We first experimented with a vaccine obtained at the height of the rise. Here it was noted that after subcutaneous injection a rise in the number of trypanosomes took place. The fact was first noted by D. Thomson, while conducting similar work on the human case of sleeping sickness. This observer asserts that an injection of a so-called vaccine—which was simply rats' blood, sterilised and mixed with normal saline—rich in the dead bodies of trypanosomes, stimulated the trypanosomes in the peripheral blood of the patient to divide; and the explanation seems to be based on the hypothesis of Dr. H. C. Ross, who holds that extracts of dead animal tissues stimulate cell division*.

In our experiments with rats infected with *T. rhodesiense*, we without exception found that when the blood of an animal rich in trypanosomes was injected subcutaneously, a rise of trypanosomes occurred during the next twenty-four hours. This rise was sometimes very sudden and exceedingly high, and if the dose was large and strong, the rise was (several times) so great as to cause the death of the animal. This is exactly in line with the treatment of disease with true bacterial vaccines, and here it is well known that if we treat say a mastitis with vaccine and give an excessive dose, we aggravate the condition. On the other hand, if a small dose was given to an infected rat, we got during the next twenty-four hours a rise, but not sufficient to kill the rat. This rise may well be represented as the negative phase, and a fall occurs usually in forty-eight to seventy-two hours, which may be considered as the positive phase.

We conclude, therefore, that the dose is important, i.e., it must not be too large, and secondly, we must at least allow an interval of one day before a second dose is administered.

In our first experiments we used six rats infected with *T. rhodesiense*, and vaccine was prepared from another rat, which was killed when the trypanosomes were at the height of a crest. We thus used a blood rich in trypanosomes, and relatively poor in anti-bodies. Three rats were kept as controls and three were treated. Two of those treated quickly succumbed, and we explain this by the fact that we gave two doses of vaccine without allowing an interval of at least twenty-four hours. We gave no time for a

* Brit. Med. Journ., June 11, 1910.

reaction to take place. The third rat, however, lived longer than the controls, and in this case, if we eliminate the fact that it may have been a more resistant animal, we had the life prolonged by the vaccine, which was given with an interval of one day between the doses.

We attempted the same line of treatment in two guinea-pigs, but here again, we cannot say that we prolonged life. We now attempted to obtain results with a blood prepared when the trypanosomes were low in the peripheral blood, i.e., we tried a condition in which one would expect to find anti-bodies, and in addition to this the blood was rich in leucocytes. The first injection, given on the eighth day, produced a fall in the trypanosomes and a rise in leucocytes which was very marked. The second injection was given on the thirteenth day, and again we obtained a fall (slight), but the leucocytes also fell. On the twentieth day we gave an injection of blood obtained from an animal when the trypanosomes were numerous; a very high rise took place and the animal died. This animal lived twenty-two days, i.e., we may conclude that we prolonged its life, and, indeed, the animal might have lived a longer period had we not on the twentieth day given too strong a dose of vaccine. We publish the chart of the last experiment (Rat 95), as it shows the rise and fall of the leucocytes in relation to the rise and fall of trypanosomes. It will be noted, if we refer to chart of Rat 95, that after inoculation a leucopenia takes place, and when trypanosomes first appear in the peripheral blood we have the leucocytes increasing. Again, it will be noted that when the trypanosomes are high in number that there tends to be a fall in the number of leucocytes, whereas when the trypanosomes are low in the peripheral blood there tends to be a marked leucocytosis. We have noticed this relation between leucocytes and trypanosomes in many cases.

We append tables of numbers of three untreated controls, viz., Rats 14, 15 and 16, and the tables of three rats treated with vaccine of a blood rich in trypanosomes, viz., Rats 42, 43 and 44, and, lastly, we append a chart of Rat 95 infected with *T. rhodesiense*. Here the first two doses of so-called vaccine were prepared from a rat when the trypanosomes were few in the peripheral blood, and we note that a fall in the numbers took place

Rat 95 T. rhodesiense. Treated with vaccine.

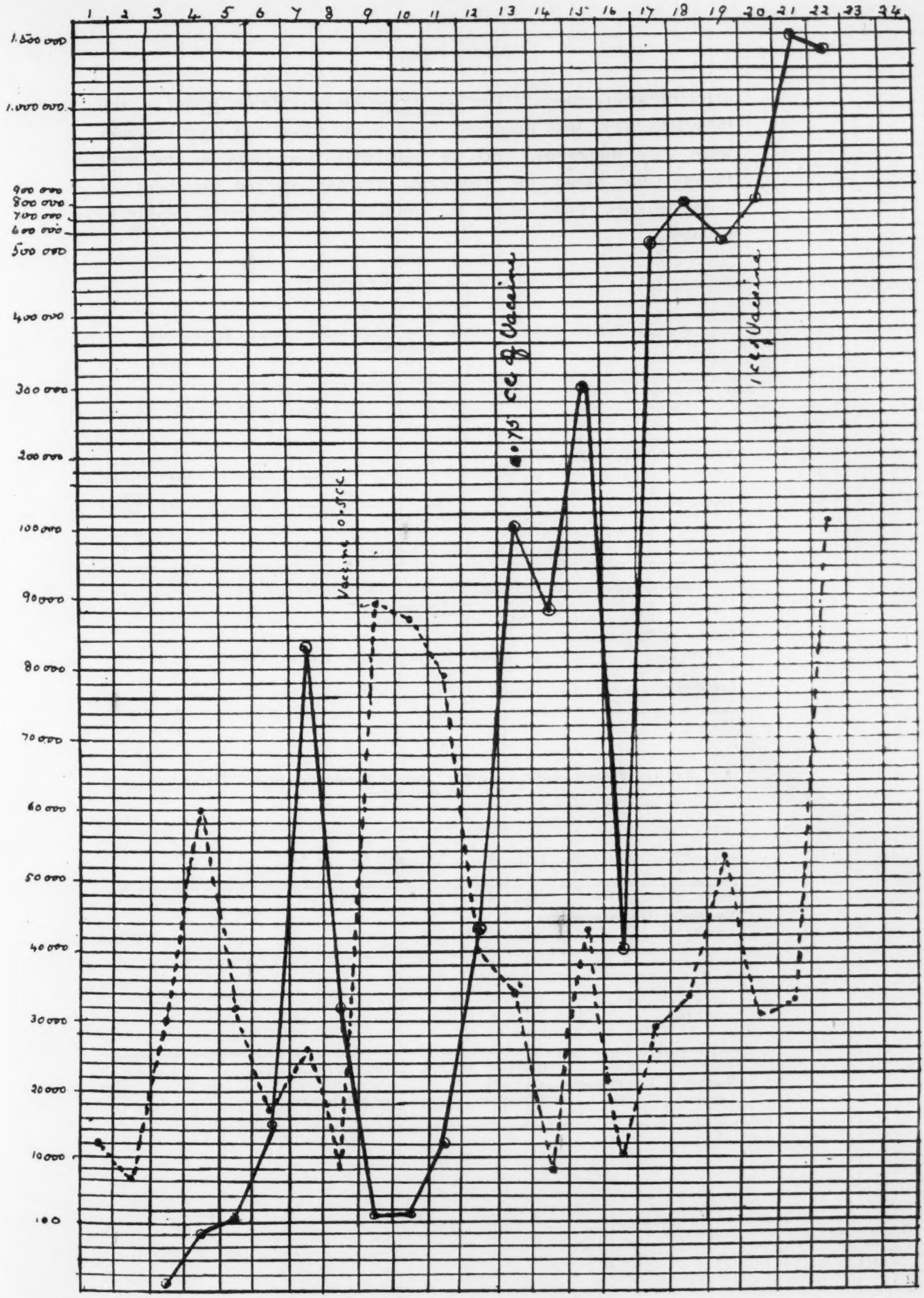

CHART 5.—Rat 95. ——— Continuous line indicates the trypanosome graph.
- - - - - - - - Dotted line indicates the graph of leucocytes. Counts made per c.mm. of peripheral blood.

after these injections. The last injection of vaccine, given on the twentieth day, was prepared from a rat when the trypanosomes were very numerous, and we note that there was in the next twenty-four hours an enormous increase in the numbers of trypanosomes, from which the animals were evidently unable to recover.

CONTROLS—NO TREATMENT

RAT 14.—Piebald, weight 127 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	1,440	45,600	66,240	7,144
Leucocytes	—	—	—	—	26,080	36,784	59,648

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	50,232	77,440	250,000	464,640	500,000	—	—
Leucocytes	—	—	—	—	—	—	—

RAT 15.—Piebald, weight 104 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	4	4,620	45,440

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	24,472	44,352	194,128	410,000	—	—	—

RAT 16.—White, weight 58 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	104	37,000	30,240
Leucocytes	—	9,204	14,000	21,060	10,468	—	—
Temp.	—	42	44	36	—	44	46

Day	8	9	10	11	12	13	—
Number of Trypanosomes per c.mm.	126,000	86,640	121,600	189,000	160,800	No count	
Leucocytes	—	—	—	—	8,416	—	—
Temp.	34	52	20	34	—	18	—

RATS TREATED WITH VACCINE

RAT 42.—White, weight 121 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	90	24,000
Vaccine	—	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	2,376	1,002	9,044	35,200	60,480	32,000	123,200
Vaccine	—	—	—	—	—	—	—

Day	15	16	17	18	19
Number of Trypanosomes per c.mm.	136,160	185,600	239,520	306,432	211,600
Vaccine	10,000,000	—	10,000,000	10,000,000	—

Rat 42—continued.

Day	20	21	—	—	—	—	—
Number of Trypanosomes per c.mm.	225,872	211,600	—	—	—	—	—
Vaccine	—	—	—	—	—	—	—

Rat 43.—White, weight 140 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6
Number of Trypanosomes per c.mm.	—	—	—	840	52,000	214,016
Vaccine	—	—	—	—	12,000,000	12,000,000

Day	7	8	9	—	—	—
Number of Trypanosomes per c.mm.	144,000	310,000	5,292	—	—	—
Vaccine	—	—	—	—	—	—

Rat 44.—Piebald, weight 237 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6
Number of Trypanosomes per c.mm.	—	—	65	35,296	42,320	248,960
Leucocytes	—	—	—	27,446	15,608	30,400
Vaccine	—	—	—	—	12,000,000	12,000,000

Day	7	8	9	10	11	—	—
Number of Trypanosomes per c.mm.	13,056	39,960	97,544	310,000	366,000	—	—
Leucocytes	43,000	—	—	—	—	—	—
Vaccine	—	—	—	—	—	—	—

III. TREATMENT IN THE COLD CHAMBER

The dimensions of the cold chamber are twelve feet long by seven feet wide by six and three-quarter feet high, and this is cooled by a refrigerator.

The lowest temperature reached in our experiments was 20° F. As the cooling apparatus was stopped during the night the temperature rose, and in the morning, before starting the machinery, reached 36° F. to 38° F., but this, of course, varied with the temperature of the atmosphere outside the chamber.

The humidity of the atmosphere in the chamber was low, and was found to vary between 50 and 60 per cent., but this also varied with the humidity outside, as the door had to be opened several times daily for the purposes of observation and to admit of feeding the animals.

For the above observations regarding humidity and temperature we are indebted to Major Williams, who compares the atmosphere of the cold chamber to that in the interior of Canada.

We were thus enabled to carry out treatment in a cold, dry atmosphere, and from personal experience we can testify to the invigorating feeling bestowed on us by a short sojourn in this chamber.

The animals in this chamber were active and took their food well, and, without doubt, seemed much better than their controls outside in the animal house.

The patient W.A., suffering from sleeping sickness contracted in Rhodesia, several times visited the cold chamber for treatment, but, unfortunately, at this time, no counts were made of the parasites in the peripheral circulation, but we have the evidence of the patient himself, who emphatically declared that he felt much better after being for some time in the cold chamber.

As the patient became worse, treatment had to be discontinued owing to the fact that the patient was considered too ill to travel from the Royal Southern Hospital to the University, and we had to resort to treatment in animals alone.

The animals used were guinea-pigs and rats, and we used two strains of trypanosomes—

(1) The *T. rhodesiense* obtained from patient W.A. (Ross and Thomson, 1910*), and

(2) An old laboratory strain of *T. gambiense*.

The essential differences between these two strains has been discussed by H. B. Fantham and J. G. Thomson in Paper on 'Enumerations in Untreated Animals suffering from Sleeping Sickness,' 1910†.

We at first selected guinea-pigs sub-inoculated with sleeping sickness, and from the tables we append it is evident that the animals were more resistant to the infection while living in the cold chamber.

T. gambiense. Old Laboratory Strain. Controls in Animal House

Animal	Incubation	Duration of life	Weight in grams
Guinea-pig 1[1]	4	28	495
Guinea-pig 2[1]	4	30	580
Guinea-pig 3[2]	4	105	534
Guinea-pig 4[2]	2	79	403

[1] Counts of these animals are not given.

[2] Daily counts of these animals are given in the paper on Enumerative Studies on Trypanosomes in Animals by Fantham and J. G. Thomson, where Guinea-pig 3 is numbered as 7 and Guinea-pig 4 is numbered as 6.

T. gambiense. Old Laboratory Strain. Treated in cold. Lowest Temp. 20° F.

Animal	Incubation	Duration of life	Weight in grams
Guinea-pig 5	19	102	648
Guinea-pig 6	6	93	562
Guinea-pig 7	16	74	623

* Proc. Roy. Soc., B, Vol. LXXXIII, pp. 187-205 (1911).

† Proc. Roy. Soc., B, Vol. LXXXIII, pp. 206-211 (1911), and Ann. Trop. Med. and Parasit., Vol. IV, pp. 417-463.

GUINEA-PIGS.—Sub-inoculated with *T. rhodesiense*

No.	Incubation	Duration of life	Weight in grams	
Guinea-pig 8 ...	13 days	120 days	350	Treated in Cold Chamber
Guinea-pig 9[1] ...	6 days	79 days	346	Treated in Animal House

[1] This is Guinea-pig 1 in paper on Enumerative Studies on Trypanosomes by Fantham and J. G. Thomson.

From the above tables it will be noted that the incubation period was delayed in all cases in the animals subjected to treatment in the cold chamber.

In the above tables Guinea-pigs 1, 2 and 3 were controls of Guinea-pigs 5 and 6.

Guinea-pig 4 was control of Guinea-pig 7, and Guinea-pig 9 was control of Guinea-pig 8.

The average incubation period of the five controls in the animal house was four days, whereas the average incubation period of the four animals treated in the cold chamber was $13\frac{1}{2}$ days.

The average duration of life in the controls was $64\frac{1}{5}$ days, whereas in the animals treated in the cold chamber the average life was $97\frac{1}{4}$ days.

In addition to this the animals in the cold were livelier, and took their food better than those in the animal house.

We can, therefore, conclude that in guinea-pigs sub-inoculated with both strains of sleeping sickness we had the incubation period delayed in the cold, and also the life of the animals prolonged when treated by cold.

We now experimented with rats. Here, again, we sub-inoculated the animals with the *T. rhodesiense* from the patient W.A., and we also sub-inoculated a series of rats with the old laboratory strain of *T. gambiense*.

CONTROLS. Old Laboratory Strain. *T. gambiense*

Number Piebald Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 23	173	6	19	2,000,000	8
Rat 27	113	5	15	60,000	7
Rat 28	101	5	17	60,000	3

COLD CHAMBER. Old Laboratory Strain. *T. gambiense*

Number Piebald Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 45	270	7	12	2,000,000	8
Rat 46	113	6	26	60,000	Fall in first 24 hours

ANIMAL HOUSE. *T. rhodesiense*

Number White Rats	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 17	62	4	8	38,000	A fall
Rat 16	58	4	13	200,000	8 divisions

COLD CHAMBER. *T. rhodesiense*

Number White Rat	Weight in grams	Incubation	Duration of life	Number of Trypanosomes inoculated	Number of divisions in first 24 hours
Rat 47	90	6	11	38,000	4 divisions

It will be noted from the above that the average incubation period of those in the cold chamber was 6·3 days, whereas the incubation period of the control was 4·8 days.

The average life in the cold was 16·3 days, and the average of those in the animal house was 14·2 days.

Rat 23 is the control of Rat 45. Rats 27 and 28 are controls of Rat 46. Rat 17 is the control of Rat 47.

We now append a table of the daily counts of the trypanosomes in the peripheral blood of animals treated in the cold. These counts were made every twenty-four hours. The incubation period is stated in days, and we define it as the period when trypanosomes are first found in one quarter cubic millimetre of peripheral blood.

The temperature in the tables is recorded according to the haemathermic scale of one of us (R.R.), where temp. = (F. − 95) × 10; F. being the temperature in degrees Fahrenheit recorded by a clinical thermometer. Temperature was taken in all cases per rectum. The counts of Guinea-pigs 1 and 2 are not given. For the counts of Guinea-pigs 3 and 4 see paper on 'Enumerative Studies on Trypanosomes,' by Fantham and J. G. Thomson, where Guinea-pig 3 is numbered as 7, and Guinea-pig 4 is numbered as 6.

GUINEA-PIG 5.—Cold Chamber. *T. gambiense*. Incubation period 19 days. Duration of life 102 days

Day	20	21	22	23	24	25	26	27
Number of Trypanosomes per c.mm.	4	16	53	28	12	24	32	40
Temp.	55	82	82	80	80	—	83	95
Weight in grams	—	—	648	—	—	—	—	—

Day	28	29	30	31	32	33	34
Number of Trypanosomes per c.mm.	92	1,060	2,208	5,568	7,108	10,476	4,806
Temp.	86	82	80	74	—	86	87
Weight in grams	—	603	—	—	—	—	—

Day	35	36	37	38	39	40	41
Number of Trypanosomes per c.mm.	4,192	160	408	200	—	300	684
Temp.	84	81	90	80	—	82	80
Weight in grams	—	608	—	—	—	—	—

Day	42	43	44	45	46	47	48
Number of Trypanosomes per c.mm.	—	—	1,040	1,520	—	—	2,880
Temp.	—	—	88	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 5—continued.

Day	49	50	51	52	53	54	55
Number of Trypanosomes per c.mm.	5,344	—	—	21,252	—	19,136	9,700
Temp.	80	—	—	86	—	82	—
Weight in grams	—	—	—	—	—	—	—

GUINEA-PIG 6.—Cold Chamber. *T. gambiense*. Incubation period six days. Duration of life 93 days. Killed accidentally by CO_2 anaesthesia

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	Tryps. present
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	—	—	—	—	7,360	18,240	12,376
Temp.	—	—	—	—	—	65	82
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Guinea-pig 6—continued.

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	17,304	18,384	29,348	9,200	4,032	3,488	2,640
Temp.	90	86	93	96	94	92	86
Weight in grams	562	—	—	—	—	—	—
Leucocytes	—	—	31,096	23,000	—	—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	2,880	880	52	144	416	1,716	3,526
Temp.	94	88	—	91	97	88	86
Weight in grams	—	—	—	—	—	—	515
Leucocytes	—	—	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	4,366	10,504	—	15,420	13,380	—	—
Temp.	86	80	—	78	75	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	840	840	—	—	2,016	4,620	—
Temp.	80	—	—	—	—	78	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	50	51	52	53	54	—	—
Number of Trypanosomes per c.mm.	—	24,128	—	66,700	57,120	—	—
Temp.	—	74	—	82	—	—	—
Weight in grams	—	—	—	—	—	—	—
Leucocytes	—	9,648	—	21,460	10,640	—	—

GUINEA-PIG 7.—Weight 623 grams. Cold Chamber. Dose of inoculation: 4,000,000. *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Leucocytes	—	—	48,000	—	—	—	—
Temp.	76	88	96	—	86	80	90
Weight in grams	623	—	—	—	—	—	594

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—
Temp.	84	90	82	—	82	80	80
Weight in grams	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	—	—	40	0	156	176	426
Leucocytes	—	—	—	—	—	—	—
Temp.	80	80	79	—	85	80	78
Weight in grams	570	—	—	—	—	—	—

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	408	600	2,688	2,800	16,640	3,584	2,688
Leucocytes	—	—	—	—	—	—	—
Temp.	82	82	80	—	—	74	88
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 7—continued.

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	600	2,448	11,852	13,104	15,708	14,900	9,680
Leucocytes	—	—	5,244	13,000	47,256	27,800	31,328
Temp.	84	—	86	—	80	74	84
Weight in grams	533	—	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	10,480	22,720	17,572	39,672	42,240	30,000	46,240
Leucocytes	24,160	15,920	33,396	15,200	—	39,672	—
Temp.	75	78	74	—	70	76	74
Weight in grams	—	—	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	195,000	108,000	36,000	46,880	39,700	55,524	68,040
Leucocytes	13,632	23,472	—	33,920	17,640	11,424	11,772
Temp.	82	53	86	—	70	70	82
Weight in grams	608	—	—	—	—	—	596

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	55,680	93,280	43,200	65,668	66,176	19,440	46,400
Leucocytes	4,000	8,500	—	16,588	—	—	—
Temp.	82	80	82	—	78	80	78
Weight in grams	—	—	—	—	—	—	—

Day	57	58	59	60	61	62	63
Number of Trypanosomes per c.mm.	52,376	70,056	28,800	44,800	21,160	37,296	62,160
Leucocytes	—	—	—	—	—	—	—
Temp.	50	80	81	—	—	95	85
Weight in grams	581	—	—	—	—	—	—

Guinea-pig 7—continued.

Day	64	65	66	67	68	69	70
Number of Trypanosomes per c.mm.	—	65,296	56,800	41,920	46,720	69,840	36,640
Leucocytes	—	—	—	—	—	—	—
Temp.	90	—	—	—	78	—	—
Weight in grams	622	—	—	—	—	—	—

Day	71	72	73	74	75	—	—
Number of Trypanosomes per c.mm.	70,664	64,584	21,672	6,880	Dead	—	—
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 8.—Cold Chamber. Weight 350 grams. Dose of inoculation: 500,000 *T. rhodesiense*. Incubation period 13 days. Duration of life 120 days.

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Leucocytes	—	35,000	—	18,304	29,456	32,208	10,208
Temp.	—	78	82	80	—	72	83
Weight in grams	—	350	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	8
Leucocytes	9,408	3,584	22,512	10,920	12,012	32,256	18,640
Temp.	83	68	80	76	—	74	82
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 8—continued.

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	2	8	4	12	16	40	8
Leucocytes	—	14,352	—	—	—	—	—
Temp.	80	82	76	77	—	70	76
Weight in grams	—	369	—	—	—	—	—

Day	22	23	24	25	26	27	28
Number of Trypanosomes per c.mm.	16	360	612	2,352	4,060	356	480
Leucocytes	—	11,760	21,692	—	30,740	—	—
Temp.	84	80	84	92	—	86	84
Weight in grams	—	387	—	—	—	—	—

Day	29	30	31	32	33	34	35
Number of Trypanosomes per c.mm.	6,440	6,720	3,192	4,320	6,216	13,376	9,760
Leucocytes	—	—	—	—	—	—	—
Temp.	86	80	81	79	—	—	92
Weight in grams	—	410	—	—	—	—	—

Day	36	37	38	39	40	41	42
Number of Trypanosomes per c.mm.	17,432	10,000	5,760	7,360	7,920	12,800	4,000
Leucocytes	—	—	—	—	—	—	—
Temp.	82	83	—	—	—	87	—
Weight in grams	—	443	—	—	—	—	—

Day	43	44	45	46	47	48	49
Number of Trypanosomes per c.mm.	7,632	2,784	40,152	9,240	8,064	10,184	14,240
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Guinea-pig 8—continued.

Day	50	51	52	53	54	55	56
Number of Trypanosomes per c.mm.	2,000	5,376	8,800	2,800	—	15,840	11,264
Leucocytes	—	—	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

CRYOTHERAPY EXPERIMENTS

Rat 23.—Piebald adult, weight 173 grams. Control in Animal House. Dose of inoculation: 2,000,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	120
Temp.	55	40	50	50	44	34	51
Leucocytes	—	—	29,000	—	—	—	—
Weight in grams	173	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	48,000	69,728	125,860	19,764	600	9,280	60,712
Temp.	45	65	50	—	50	66	38
Leucocytes	—	—	—	17,712	—	—	—
Weight in grams	167	—	—	—	—	—	—

Day	15	16	17	18	19	—	—
Number of Trypanosomes per c.mm.	130,000	142,000	100,000	250,000	164,000	—	—
Temp.	34	40	55	—	11	—	—
Leucocytes	—	—	—	—	—	—	—
Weight in grams	160	—	—	—	—	—	—

RAT 27.—White adult, weight 113 grams. Control in Animal House. Inoculation dose: 60,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	120	14,310
Temp.	61	49	32	12	44	—	5
Leucocytes	—	—	—	—	—	—	—
Weight in grams	113	—	112	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	101,740	146,880	69,400	23,276	55,224	67,096	57,552
Temp.	40	44	36	36	35	—	—
Leucocytes	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

Day	15	—	—	—	—	—	—
Number of Trypanosomes per c.mm.	6,120	Dead	—	—	—	—	—
Temp.	—	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—
Weight in grams	—	—	—	—	—	—	—

RAT 28.—White adult, weight 101 grams. Control in Animal House. Inoculation dose: 60,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	256	2,808
Temp.	59	70	43	42	40	—	56
Weight in grams	101	—	98	—	—	—	—

Rat 28—continued.

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	4,416	3,648	24,904	90,509	118,700	143,520	270,000
Temp.	44	42	34	26	24	—	40
Weight in grams	—	—	—	—	—	—	—

Day	15	16	17	18	—	—	—
Number of Trypanosomes per c.mm.	100,000	200,600	6,048	Dead	—	—	—
Temp.	78	42	—	—	—	—	—
Weight in grams	—	—	88	—	—	—	—

CRYOTHERAPY EXPERIMENTS

Rat 45.—Cold Chamber. Lowest temp. 20° F. Weight 270 grams. Dose of inoculation: 2,000,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	—
Temp.	54	70	60	—	56	52	60
Weight in grams	270	—	—	—	—	—	—
Leucocytes	—	—	45,000	—	—	—	—

Day	8	9	10	11	12	—	—
Number of Trypanosomes per c.mm.	92	24,012	480	430	1,100	—	—
Temp.	50	54	57	—	0	—	—
Weight in grams	246	—	—	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

RAT 46.—Cold Chamber. Weight 113 grams. Dose of inoculation: 60,000 *T. gambiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	3,608
Temp.	60	60	50	78	60	—	63
Weight in grams	113	—	111	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	3,456	2,352	8,640	2,400	7,100	3,104	13,024
Temp.	62	50	62	60	56	—	—
Weight in grams	—	—	+	—	—	—	—
Leucocytes	—	—	—	—	—	—	—

Day	15	16	17	18	19	20	21
Number of Trypanosomes per c.mm.	40,120	63,480	11,448	61,560	16,416	28,188	46,980
Temp.	40	52	50	55	—	—	70
Weight in grams	—	—	125	—	—	—	—
Leucocytes	—	14,260	—	12,236	26,432	22,736	23,160

Day	22	23	24	25	26	27	—
Number of Trypanosomes per c.mm.	85,120	82,080	393,120	350,000	225,680	Dead	—
Temp.	68	63	34	58	50		
Weight in grams	—	—	—	—	—	—	—
Leucocytes	8,136	20,832	23,000	25,688	—		—

RAT 17.—White, weight 62 grams. Animal House. Dose of inoculation : 38,400 *T. rhodesiense*

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm.	—	—	—	—	1,500	500	1,380	4,028
Leucocytes	—	—	—	—	38,700	—	—	—
Temp.	—	50	30	56	50	—	—	70

RAT 16.—White, weight 58 grams. Animal House. Dose of inoculation : 200,000 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	104	37,000	30,240
Leucocytes	—	9,204	14,000	21,060	10,468	30,800	28,000
Temp.	—	42	44	36	—	44	46

Day	8	9	10	11	12	13	—
Number of Trypanosomes per c.mm.	126,000	86,640	121,600	189,000	160,800	No count	
Leucocytes	18,584	27,360	16,340	38,124	8,416	—	—
Temp.	34	52	20	34	—	18	—

RAT 47.—Cold Chamber. Weight 90 grams. Dose of inoculation : 38,400 *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	—	—	5,184
Leucocytes	—	—	—	—	—	—	—
Temp.	—	50	45	50	40	—	—

Day	8	9	10	11	—	—	—
Number of Trypanosomes per c.mm.	82,818	230,000	106,000	330,000	—	—	—
Leucocytes	—	—	—	—	—	—	—
Temp.	40	66	—	36	—	—	—

The conclusions to be drawn from the above experiments are therefore in favour of treatment in the cold. The resistance of the animal is evidently raised, for the incubation period is delayed, and the animal lives longer in the cold. In favour of the cold also, we have the evidence of the patient, W.A., who said he felt better when in the cold chamber. What the physiological action of cold is we are not prepared in our present state of knowledge to state, but we certainly think that a person warmly clad is beneficially acted upon by a cold, dry atmosphere, and we would suggest that patients suffering from sleeping sickness would be greatly benefited by a sojourn, say, in Canada or Switzerland. The cold seems to be a valuable therapeutic agent in treatment.

We must acknowledge the fact that much more must be done in this investigation before definite conclusions are drawn, but the results have been so encouraging that we think they ought to be made known at once.

IV. X-RAYS

A young piebald rat, weighing 77 grams was inoculated with the Rhodesian strain of trypanosomes.

Here we have to thank Dr. Morgan, in charge of the Electrical Department at the Royal Southern Hospital, who kindly advised us in the administration of the rays. The exposures to the rays were given by Miss Wells, an experienced worker, under the supervision of Dr. Morgan. The rat lived fifteen days, and we can therefore say that this animal's life was prolonged, when compared with the average life of twenty-two untreated rats, which was 11·3 days.

In spite of five exposures, each of twenty minutes duration, during which period the whole body of the rat was exposed to the direct action of the rays, the trypanosomes remained lively and increased steadily in number in the peripheral circulation.

The rays were not, therefore, trypanocidal in the exposures given by us, but, curiously enough, the life of the animal was prolonged.

We append table:—

RAT 50.—Piebald, weight 77 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7	8
Number of Trypanosomes per c.mm.	—	—	60	32,256	227,840	305,376	287,776	350,00
X-rays for 20 minutes	—	—	—	—	X-rays	—	X-rays	—

Day	9	10	11	12	13	14	15
Number of Trypanosomes per c.mm.	252,320	350,000	350,000	410,000	366,000	430,000	323,840
X-rays for 20 minutes	—	—	X-rays	—	X-rays	—	X-rays

The animal always seemed to brighten up during the exposure to the rays. The trypanosomes, it will be noted, increased in numbers in the peripheral circulation after each exposure, and we would conclude that there was no destruction of the trypanosomes. This is exactly in line with the experiments made by one of us (R.R.) several years ago, who found that exposure of the trypanosomes *in vitro* to the influence of X-ray had no trypanocidal action.

V. LEUCOCYTIC EXTRACT

This experiment was carried out at the suggestion of Dr. Moore Alexander, Pathologist to the Royal Southern Hospital, and he very kindly prepared the extract for us.

A white rat, weighing 120 grams, was inoculated with the Rhodesian strain of trypanosomes, and the disease was allowed to incubate. On the twelfth day, when the trypanosomes numbered 89,000 per c.mm., we injected subcutaneously 0·5 c.c. of leucocytic extract. The following day the trypanosomes numbered 220,800 per c.mm., and the leucocytes rose from 8,160 per c.mm. to 10,764 per c.mm. The animal lived fourteen days. We cannot, therefore, draw conclusions here, and much further work will have to be undertaken before we conclude as to the value of leucocytic extract in trypanosomiasis.

We append table:—

RAT 51.—White, weight 120 grams. Dose of inoculation: 200,000 Trypanosomes *T. rhodesiense*

Day	1	2	3	4	5	6	7
Number of Trypanosomes per c.mm.	—	—	—	—	48	27,720	76,960
Leucocytes	—	—	—	5,680	—	—	19,040
Leucocytic extract	—	—	—	—	—	—	—

Day	8	9	10	11	12	13	14
Number of Trypanosomes per c.mm.	11,760	11,960	66,360	13,272	89,000	220,800	472,000
Leucocytes	—	40,296	—	—	8,160	10,764	—
Leucocytic extract	—	—	—	—	0·5 c.c.	—	—

AUTO-AGGLUTINATION OF RED BLOOD CELLS IN TRYPANOSOMIASIS*

BY

WARRINGTON YORKE, M.D.

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(*From the Runcorn Research Laboratories of the Liverpool School of Tropical Medicine.*)

CONTENTS

INTRODUCTION

It is now a well-recognised fact that the erythrocytes in fresh preparations of the blood of sleeping sickness cases and animals infected with trypanosomiasis frequently exhibit a more or less marked degree of agglutination. Attention was first drawn to this phenomenon in 1898 by Kanthack, Durham and Blandford,† who found that the red blood cells of animals infected with nagana,

* Reprinted from Proc. Roy. Soc., B. Vol. LXXXIII, p. 238.

† 'On Nagana or Tsetse Fly Disease,' Roy. Soc. Proc., 1898, Vol. LXIV, p. 100.

instead of forming rouleaux, tended to clump together into masses and to lose their outlines.

More recently Christy* (1904), Dutton and Todd† (1905), Martin, Lebœuf and Roubaud‡ (1906-8), and others have described a similar condition in fresh preparations of the blood of patients suffering from sleeping sickness.

Dutton and Todd,§ referring to this condition of the blood, wrote: 'Only once have we had the opportunity of observing a 'patient (European) from whose blood trypanosomes, once present, 'have finally disappeared.' In this instance auto-agglutination of the red cells disappeared with the parasites.

Later, it has been noted by many investigators‖ that the phenomenon gradually disappears in animals after the successful drug treatment of the disease.

In spite of the fact that auto-agglutination of the red cells in trypanosomal infections has attracted so much attention, very little work appears to have been done with a view to determining the nature of the changes in the blood which lead to its manifestation. Most of the workers have contented themselves with simply recording the presence of the phenomenon. So far as I have been able to ascertain from a search of the literature, only two authors mention any details regarding the mechanism of its production.

Kanthack, Durham and Blandford stated that the serum of the blood of animals exhibiting auto-agglutination, when added to normal blood of the same species of animal, caused the red corpuscles to clump together. On the other hand, at a recent meeting of the Society of Tropical Medicine and Hygiene, in the

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* 'Sleeping Sickness,' British Medical Journal, 1904, p. 1456.

† 'Gland Puncture in Trypanosomiasis,' Memoir of the Liverpool School of Tropical Medicine, 1905, No. 16, p. 99.

‡ Rapport de la Mission d'Études de la Maladie du Sommeil au Congo Français, 1906-8, p. 281.

§ *Loc. cit.*

‖ Thomas and Breinl, 'Pathology and Treatment of Trypanosomiasis,' Memoir of the Liverpool School of Tropical Medicine, 1905, No. 16.

Mesnil, Nicolle, and Aubert, 'Recherches sur le Traitement des Infections Expérimentales à *T. gambiense*,' Annales de l'Institut Pasteur, 1907, Vol. XXI, p. 1.

Laveran and Thiroux, 'Sur le Traitement des Trypanosomiases,' Bulletin Soc. Path. Exot., 1908, p. 28.

Martin and Darré, 'Sur les Symptômes Nerveux du Début de la Maladie du Sommeil,' Bulletin Soc. Path. Exot., 1908, p. 15.

discussion following Dr. Bagshawe's paper on 'Recent Advances in our Knowledge of Sleeping Sickness,' Breinl* remarked that with regard to its (auto-agglutination) mechanism he had not, up to the present, been able to arrive at any definite conclusions. All his attempts to isolate a haemagglutinin had failed, and he had not been able to demonstrate either an iso- or an auto-agglutinin. Dr. Nierenstein† had shown that in trypanosomiasis a definite increase of the acidity of the blood occurred. This was most probably due to an increase of the amino-acids in the blood, and Breinl and Nierenstein inclined to the view that auto-agglutination was caused by this excess of amino-acids. Unfortunately, they had not so far been able to find a definite proof of the correctness of this conception.

The results obtained by these investigators are thus somewhat conflicting, for whereas Kanthack, Durham, and Blandford found an iso-agglutinin in the serum of certain animals infected with *T. brucei*, Breinl was unable to demonstrate the existence of either an auto- or an iso-agglutinin.

EXAMINATION OF SERUM OF INFECTED ANIMALS FOR AUTO-AGGLUTININ

Having under observation a number of animals, the blood of which when examined in cover-slip preparations exhibited the phenomenon of auto-agglutination to a greater or less degree, it was decided to perform experiments with a view to investigating the mechanism of its production.

Technique.—The blood was collected in a solution containing 1 per cent. sodium citrate and 0·9 per cent. sodium chloride in distilled water. The blood was then centrifugalised and the corpuscles washed three times in normal saline solution. A 5 per cent. suspension of the washed red cells was then made in normal saline. Another sample of the same blood was collected and allowed to clot and the serum subsequently freed from the clot by centrifugalisation.

* Trans. Soc. Trop. Med. and Hygiene, 1909, p. 29.

† 'Observations on the Acidity and Alkalinity of the Blood in Trypanosome Infections,' Annals of Tropical Medicine and Parasitology, 1908, p. 227

Equal amounts of the serum and emulsion of red corpuscles were then mixed together and drawn up into a capillary tube which was placed vertically in the incubator at 37° C.

The results of such experiments may be briefly summarised. With the exception of a few cases in which there appeared to be a trace of agglutination they were all negative. Similar negative results were obtained when the sera were examined for the presence of iso-agglutinins and also when the serum was replaced by citrated plasma.

At first one was somewhat at a loss to account for these apparently conflicting results, as even the sera of animals where the cover-slip preparations exhibited the most marked auto-agglutination of the red cells gave invariably negative results when examined for auto- and iso-agglutinins by this method.

Later it was observed that when the blood from one of the infected animals was allowed to flow from a vein of the ear into a watch-glass containing a small amount of citrated saline solution, the red cells quickly sank to the bottom in little clumps, producing in a marked degree the sandy appearance described by Dutton and Todd.* When, however, the watch-glass and salt solution were warmed to 37° C. and the blood dropped in as previously, this appearance did not result; the red cells remained suspended for a considerable time and only subsided gradually, as in the case of normal blood. So long as the temperature of the watch-glass and salt solution was kept at 37° C., no agglutination resulted, but as soon as the temperature was allowed to fall to about 18° C., the red cells ran together into clumps and the typical sandy appearance was obtained.

These observations served to indicate that temperature played an important *rôle* in the development of the phenomenon. Accordingly, the previous experiment was repeated, but on this occasion three sets of tests were made: the first were placed in the incubator at 37° C., the second were left at laboratory temperature (18° to 21° C.), the last were kept in the ice chest at 0° C. Even at the end of five minutes a certain degree of agglutination of the red cells was noticeable in some of the tubes which had been placed in the ice

* *Loc. cit.*

chest, whilst in fifteen minutes the red cells in most of them were completely agglutinated, numerous clumps of various sizes being visible in the clear serum. The reaction was also distinct in many of the tubes kept at the laboratory temperature, but it was neither so marked, nor did it occur so quickly as in those subjected to the lower temperature. As before, no agglutination—or only occasionally a trace—was observable in the tests which had been placed in the incubator at 37° C.

A large number of similar experiments were subsequently performed with the blood of monkeys, donkeys, goats, dogs, rabbits, guinea-pigs, and rats infected with various strains of trypanosomes. As a rule, it was found that a marked degree of agglutination only resulted when the temperature of the mixture of serum and red cells was lowered. Very exceptionally slight traces of agglutination were also seen in the tests carried out at 37° C., but these could not be compared with the intensity of the reaction at low temperatures.

Quite frequently a well-marked auto-agglutination was found to occur, at 0° C., in the control tests made with the blood of normal animals. I shall return to this important point later.

Iso-agglutination.—A series of experiments were undertaken with a view to ascertaining whether the sera of those infected animals which possessed the property of agglutinating their own red cells were also capable of producing agglutination of the erythrocytes of other animals of the same species. In every case where auto-agglutination was present, iso-agglutination was found to occur when the serum of the infected animal was added to the red cells of another animal (either normal or infected) of the same species.

Analogous results were obtained with the blood of a case of human trypanosomiasis in Major Ross's clinic in Liverpool. Cover-slip preparations of the blood of this case exhibited a certain degree of auto-agglutination during the six months he was under observation. The following table gives the results of an examination of his blood, drawn ten days before his death, for auto- and iso-agglutinins.

TABLE I.—Examination of Sleeping Sickness Serum for Auto- and Iso-agglutinin

Equal volumes of serum and red blood cell suspension used.		Temp.	Result
5 per cent. suspension of washed erythrocytes in normal saline solution	Source from which serum was obtained		
		° C.	
Sleeping Sickness	Sleeping Sickness	37	No agglutination in 30 mins.
		18	Marked ,, 30 ,,
		0	Complete ,, 10 ,,
Normal individual A ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Complete ,, 30 ,,
		0	,, ,, 10 ,,
Normal individual B ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Marked ,, 30 ,,
		0	Complete ,, 10 ,,
Normal individual C ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Marked ,, 30 ,,
		0	Complete ,, 10 ,,
Normal individual C ...	Normal individual C	37	No agglutination in 30 ,,
		18	,, ,, 30 ,,
		0	,, ,, 30 ,,
Normal individual C ...	Normal individual B	37	No agglutination in 30 ,,
		18	,, ,, 30 ,,
		0	Marked ,, 30 ,,

SPONTANEOUS AGGLUTINATION OF THE RED CELL SUSPENSIONS

On rare occasions it was found that the 5 per cent. suspension of red cells which had been washed three times in large volumes of 0·9 per cent. sodium chloride solution underwent a spontaneous agglutination in the entire absence of serum. Indeed, in one or two instances, where the animals exhibited an extreme degree of auto-agglutination, some difficulty was experienced in obtaining an even suspension of the erythrocytes. The probable explanation of this spontaneous agglutination is that it was due to the absorption of agglutinin from the plasma by the red cells immediately after the blood was shed into the cold citrated saline solution.

This difficulty was obviated by collecting the blood in warm citrate solution, and then rapidly centrifugalising and decanting off the citrated plasma. The red cells were then washed thrice in warm normal saline solution. Suspensions prepared in this way exhibited no tendency to spontaneous clumping.

It might be mentioned in this connection that Klein* has succeeded in obtaining agglutinating solutions by grinding up with quartz sand the well-washed erythrocytes of certain animals (rabbit, dog, hen, and guinea-pig). These extracts sometimes agglutinated the red cells of other animals, and frequently also the erythrocytes of the same kind of animal, and even those of the same animal.

ABSORPTION OF AGGLUTININ BY RED CELLS

Experiment.—To one volume of the citrated plasma of Rabbit 896 (infected with *T. dimorphon*), which caused great agglutination when added to its own red cells and to those of normal rabbits, were added five volumes of the undiluted well-washed red cells of the same animal. The mixture was then divided into two equal portions, A and B. A was placed in the incubator at 37° C. and B in the ice chest at 0° C. At the end of three hours the extracted plasmas were separated from the red cells by centrifugalisation, and were examined for auto- and iso-agglutinins.

TABLE II.

Equal volumes of extracted plasma and erythrocyte suspension used. Temp. of experiment 0° C.		Result
5 per cent. suspension of washed erythrocytes in normal saline solution.	Extracted plasma	
Rabbit 896	A	Complete agglutination in 10 mins.
,, ...	B	No agglutination in 60 mins.
Normal rabbit	A	Complete agglutination in 10 mins.
,, ...	B	Slight agglutination in 60 mins.

The plasma which had been in contact with the red cells at 0° C. had almost completely lost its agglutinating action, whilst the other

* 'Beitrage zur Kenntniss der Agglutination rother Blutkörperchen,' Wien. Klin. Woch., 1902, No. 16, p. 413.

TABLE I.—Examination of Sleeping Sickness Serum for Auto- and Iso-agglutinin

Equal volumes of serum and red blood cell suspension used.		Temp.	Result
5 per cent. suspension of washed erythrocytes in normal saline solution	Source from which serum was obtained		
		° C.	
Sleeping Sickness	Sleeping Sickness	37	No agglutination in 30 mins.
		18	Marked ,, 30 ,,
		0	Complete ,, 10 ,,
Normal individual A ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Complete ,, 30 ,,
		0	,, ,, 10 ,,
Normal individual B ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Marked ,, 30 ,,
		0	Complete ,, 10 ,,
Normal individual C ...	Sleeping Sickness	37	No agglutination in 30 ,,
		18	Marked ,, 30 ,,
		0	Complete ,, 10 ,,
Normal individual C ...	Normal individual C	37	No agglutination in 30 ,,
		18	,, ,, 30 ,,
		0	,, ,, 30 ,,
Normal individual C ...	Normal individual B	37	No agglutination in 30 ,,
		18	,, ,, 30 ,,
		0	Marked ,, 30 ,,

SPONTANEOUS AGGLUTINATION OF THE RED CELL SUSPENSIONS

On rare occasions it was found that the 5 per cent. suspension of red cells which had been washed three times in large volumes of 0·9 per cent. sodium chloride solution underwent a spontaneous agglutination in the entire absence of serum. Indeed, in one or two instances, where the animals exhibited an extreme degree of auto-agglutination, some difficulty was experienced in obtaining an even suspension of the erythrocytes. The probable explanation of this spontaneous agglutination is that it was due to the absorption of agglutinin from the plasma by the red cells immediately after the blood was shed into the cold citrated saline solution.

This difficulty was obviated by collecting the blood in warm citrate solution, and then rapidly centrifugalising and decanting off the citrated plasma. The red cells were then washed thrice in warm normal saline solution. Suspensions prepared in this way exhibited no tendency to spontaneous clumping.

It might be mentioned in this connection that Klein* has succeeded in obtaining agglutinating solutions by grinding up with quartz sand the well-washed erythrocytes of certain animals (rabbit, dog, hen, and guinea-pig). These extracts sometimes agglutinated the red cells of other animals, and frequently also the erythrocytes of the same kind of animal, and even those of the same animal.

ABSORPTION OF AGGLUTININ BY RED CELLS

Experiment.—To one volume of the citrated plasma of Rabbit 896 (infected with *T. dimorphon*), which caused great agglutination when added to its own red cells and to those of normal rabbits, were added five volumes of the undiluted well-washed red cells of the same animal. The mixture was then divided into two equal portions, A and B. A was placed in the incubator at 37° C. and B in the ice chest at 0° C. At the end of three hours the extracted plasmas were separated from the red cells by centrifugalisation, and were examined for auto- and iso-agglutinins.

TABLE II.

Equal volumes of extracted plasma and erythrocyte suspension used. Temp. of experiment 0° C.		Result
5 per cent. suspension of washed erythrocytes in normal saline solution.	Extracted plasma	
Rabbit 896	A	Complete agglutination in 10 mins.
,, ...	B	No agglutination in 60 mins.
Normal rabbit	A	Complete agglutination in 10 mins.
,, ...	B	Slight agglutination in 60 mins.

The plasma which had been in contact with the red cells at 0° C. had almost completely lost its agglutinating action, whilst the other

* 'Beitrage zur Kenntniss der Agglutination rother Blutkörperchen,' Wien. Klin. Woch., 1902, No. 16, p. 413.

portion, B, which had been placed in the incubator, had retained its auto- and iso-agglutinins intact.

The following observation furnishes additional proof of the capacity of erythrocytes to absorb haemagglutinin in the cold. Specimens of the blood of a number of infected animals were collected in sterile tubes and placed immediately in the ice chest to clot. After six hours the sera were separated from the clots by centrifugalisation and were examined for auto- and iso-agglutinins. In several cases the results were negative, no auto- or iso-agglutination being observed. In other instances the red cells were found to be clumped to a greater or less degree. The amount of agglutinin present in these sera was then compared with that occurring in the citrated plasma of the same animals which had been separated from the red cells at 37° C. The following procedure was adopted:—The serum and plasma were diluted with gradually increasing amounts of 0·9 per cent. sodium chloride solution, and the degree of dilution observed at which they no longer caused complete agglutination of a given volume of the red cell suspension in a stated time.

The results obtained with one of the animals (Rabbit 1035, infected with *T. brucei*) are given in tabular form. It is to be observed that there was at least five or six times as much agglutinin in the plasma which had been separated from the red cells at 37° C. as in the serum obtained from the blood which had been allowed to clot in the ice box.

The sera of the other animals all showed a considerable deficiency in agglutinin as compared with that present in the plasma of the same animals.

When examining the blood for auto-agglutination it was found that the strongest reactions were obtained by dropping the blood into a very small quantity of warm citrated saline solution, and then separating the plasma from the red cells as speedily as possible with the centrifuge. A perhaps even more satisfactory method, and one in which the dilution of the plasma by the citrate is avoided, is to use the defibrinated plasma obtained by shaking the blood at a sustained temperature of between 37° and 40° C. in a bottle containing a few glass beads.

TABLE III.—Diminution of Amount of Auto- and Iso-agglutinin in Serum obtained from Blood which was allowed to Clot at 0° C.

Equal volumes of red cell suspension and diluted serum or plasma used. Temp. of experiment 0° C.		
5 per cent. suspension of washed red cells of Rabbit 1035 (infected with *T. brucei*) and of normal rabbit	Serum of Rabbit 1035 obtained by allowing the blood to clot at 0° C. Plasma of Rabbit 1035 separated from the red cells at 37° C.	Result
Rabbit 1035	Undiluted serum	Complete auto-agglutination in 15 mins.
	,, plasma	,, ,, 10 ,,
Normal rabbit	,, serum	Complete iso-agglutination 10 ,,
	,, plasma	,, ,, 10 ,,
Rabbit 1035	Serum diluted with an equal volume of 0·9 per cent. NaCl solution ...	Complete auto-agglutination in 20 ,,
	Plasma ,, ,, ,, ...	,, ,, 10 ,,
Normal rabbit	Serum ,, ,, ,, ...	Complete iso-agglutination 10 ,,
	Plasma ,, ,, ,, ...	,, ,, 10 ,,
Rabbit 1035	Serum diluted with 2 volumes of 0·9 per cent. NaCl solution	Complete auto-agglutination in 30 ,, *
	Plasma ,, ,, ,, ...	,, ,, 15 ,,
Normal rabbit	Serum ,, ,, ,, ...	Complete iso-agglutination 15 ,,
	Plasma ,, ,, ,, ...	,, ,, 10 ,,
Rabbit 1035	Serum diluted with 3 volumes of 0·9 per cent. NaCl solution	Partial auto-agglutination in 30 ,,
	Plasma ,, ,, ,, ...	Complete ,, 30 ,,
Normal rabbit	Serum ,, ,, ,, ...	Complete iso-agglutination 20 ,, †
	Plasma ,, ,, ,, ...	,, ,, 20 ,,
Rabbit 1035	Serum diluted with 4 volumes of 0·9 per cent. NaCl solution	Trace auto-agglutination in 30 ,,
	Plasma ,, ,, ,, ...	Complete ,, 30 ,,
Normal rabbit	Serum ,, ,, ,, ...	Very marked iso-agglutination 30 ,,
	Plasma ,, ,, ,, ...	Complete ,, 20 ,,
Rabbit 1035	Serum diluted with 6 volumes of 0·9 per cent. NaCl solution	No agglutination in 30 ,,
	Plasma ,, ,, ,, ...	Complete auto-agglutination 30 ,,
Normal rabbit	Serum ,, ,, ,, ...	Slight iso-agglutination 30 ,,
	Plasma ,, ,, ,, ...	Complete ,, 20 ,,
Rabbit 1035	Serum diluted with 9 volumes of 0·9 per cent. NaCl solution	No agglutination in 30 ,,
	Plasma ,, ,, ,, ...	Complete auto-agglutination 30 ,,
Normal rabbit	Serum ,, ,, ,, ...	No iso-agglutination 30 ,,
	Plasma ,, ,, ,, ...	Complete iso-agglutination 20 ,,
Rabbit 1035	Plasma diluted with 12 volumes of 0·9 per cent. NaCl solution	Complete auto-agglutination in 30 ,, ‡
Normal rabbit	,, ,, ,, ...	,, iso-agglutination 30 ,,
Rabbit 1035	Plasma diluted with 14 volumes of 0·9 per cent. NaCl solution	Partial auto-agglutination in 30 ,,
Normal rabbit	,, ,, ,, ...	Complete iso-agglutination 30 ,, §
Rabbit 1035	Plasma diluted with 19 volumes of 0·9 per cent. NaCl solution	No agglutination in 30 ,,
Normal rabbit	,, ,, ,, ...	Slight iso-agglutination 30 ,,

* Denotes the end point of the auto-reaction of serum.
‡ ,, ,, ,, ,, plasma.
† Denotes the end point of iso-reaction of serum.
§ ,, ,, ,, ,, plasma.

Well marked agglutination was frequently observed when the plasma obtained in this manner was added to a 5 per cent. suspension of red cells, whilst either a negative or only slightly positive result was obtained with the serum derived from blood which had clotted at laboratory temperature or in the ice chest.

REVERSIBILITY OF THE REACTION

In view of the fact that red cells absorb agglutinin to a much greater extent at low temperatures and only slightly at higher temperatures (37° to 40° C.), the question arises as to whether the absorption of agglutinin belongs to the group of reactions which have been designated 'reversible.'* In other words, will raising the temperature of the agglutinated masses of red cells cause the clumps to disintegrate into their corpuscular elements?

Experiment.—One volume of red corpuscles of Rabbit 1022 (infected with *T. gambiense*) was added to twenty volumes of the defibrinated plasma of the same animal. After an hour's sojourn in the ice chest complete agglutination of the erythrocytes was found to have occurred. After stirring the clumps up thoroughly with a glass rod a small drop of the suspension was placed on a cover-slip and a hanging drop preparation made. On examining with the microscope large masses of agglomerated red blood cells and also considerable rouleaux formation were seen. The mixture of clumped red cells and defibrinated plasma was now placed in the incubator at 37° C. In about fifteen minutes the clumps were no longer visible, and the erythrocytes appeared to be evenly suspended throughout the fluid. A hanging drop preparation was made on a warm slide and cover-slip. As long as the temperature was maintained at 37° C. there was no tendency to agglutination. When the suspension was again cooled to 0° C. agglutination of the red cells reappeared after a few minutes.

From this and similar experiments it follows that the reaction is reversible, the phenomenon disappearing on warming and reappearing on cooling.

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* Arrhenius, Immuno-Chemistry, ch. 2.

AUTO-AGGLUTINATION IN THE BLOOD OF NORMAL ANIMALS

Attention has already been drawn to the fact that a certain amount of auto-agglutination was frequently observed in the control tests of normal blood. Some years ago Klein* found auto-agglutinin to be present in the serum of a number of normal horses.

Landsteiner† demonstrated the existence of a similar substance in the blood of rabbits, horses, dogs, and cattle.

Other writers, on the contrary, deny the existence of auto-agglutinin in normal blood. Dudgeon‡ in a recent paper states that auto-agglutination does not occur in normal human blood.

It was decided to re-investigate this subject more fully, using the blood of a considerable number of normal animals of different kinds.

Technique.—The blood was obtained from a convenient vein, and the defibrinated plasma separated from the red corpuscles at 37° C. in the manner already described. The red corpuscles were washed three times in warm saline solution, and finally a 5 per cent. suspension made in 0·9 per cent. sodium chloride solution. Equal volumes of the defibrinated plasma and red cell suspension were drawn up together into three fine pipettes which were then subjected to a temperature of 0°, 15°, and 37° C. respectively. The pipettes were kept in the vertical position, and the contents examined for auto-agglutination with the aid of a lens from time to time. It was found in the majority of cases that the test could not well be continued for longer than one hour, owing to the fact that in most cases the erythrocytes had subsided to a marked degree after the lapse of this period. At times the citrated plasma was substituted for the defibrinated plasma. The same precautions regarding temperature were taken, and only very small amounts of citrate solution (not more than a tenth of the volume of plasma)

* 'Beiträge zur Kenntniss der Agglutination rother Blutkörperchen,' Wien. Klin. Woch., 1902, No. 16, p. 413.

† 'Ueber Beziehungen zwischen dem Blutserum und den Körperzellen,' Münch. Med. Woch., 1903, No. 42.

‡ 'On the Presence of Haemagglutinins, etc., in the Blood obtained from Infectious and Non-Infectious Diseases in Man,' Roy. Soc. Proc., 1909, B, Vol. LXXXI, p. 207.

employed. The plasma obtained in this way frequently clotted, but the process was sufficiently retarded to permit of the previous separation of the red corpuscles. No appreciable difference between the agglutinating action of the defibrinated and citrated plasma was observed.

The results of this investigation of normal blood for auto-agglutinin may be summarised by stating that small quantities of auto-agglutinin were found to be present in the blood of rabbits (14), guinea-pigs (4), goats (3), dogs (2), horses (4), donkeys (2), monkeys (*Macacus rhesus*) (2), and *Cercopithecus callitrichus* (2). Sometimes, especially in goats and guinea-pigs, the amount present was exceedingly small, and considerable care was necessary to demonstrate its existence. In these cases a larger volume of serum was used in proportion to the amount of red cells and the reaction allowed to proceed for a longer period. It is to be observed that clumping of the erythrocytes only occurred in the tests carried out at low temperatures, and not in those subjected to a temperature of 37° C.

RELATION OF AUTO-AGGLUTININ OF NORMAL BLOOD TO THAT PRESENT IN THE BLOOD OF ANIMALS INFECTED WITH TRYPANOSOMES

In this connection it may be remarked that in the blood of infected animals there exists a considerable excess of auto-agglutinin beyond that present in the blood of normal animals. It was found that diluting the defibrinated plasma of normal blood with twice its volume of normal saline solution usually sufficed to destroy its agglutinating action. On the other hand, it was often possible to dilute the infected plasma 15- or 20-fold, and still obtain complete agglutination of the erythrocyte suspension.

EFFECT OF HEAT ON AUTO-AGGLUTININ

Different portions of the defibrinated plasma of normal and infected animals were heated in a water bath to 58° C. and 70° to 72° C. respectively for twenty minutes. Heating to 58° C. was found not to destroy auto-agglutinin, whereas plasma which had been subjected to a temperature of 70° C. for twenty minutes had completely lost this property.

SIGNIFICANCE OF THE PHENOMENON IN TRYPANOSOMAL INFECTIONS

The question of the mechanism of production of auto-agglutination in trypanosomal infections is one which has frequently been discussed, but as yet no satisfactory explanation has been offered. With reference to this question it appears to me that two theories might be advanced to explain the development of an excess of auto-agglutinin in this disease.

It has long been recognised that the blood of men and the lower animals suffering from trypanosomaisis is frequently very anaemic. Both the percentage of haemoglobin and the number of red corpuscles per cubic millimetre fall to a low level. This is particularly the case in the last stages of the disease. Conceivably auto-agglutinin might develop in the plasma as a result of auto-inoculation of an animal resulting from the destruction of its own erythrocytes.

There are, however, many considerations which operate against this view. In the first place I have found no constant relation between the development of anaemia and auto-agglutination of the red cells. By the aid of systematic haemocrit examinations of the blood of recently infected animals it was observed that auto-agglutination was usually pronounced for a considerable period before any marked fall of the haemocrit value had occurred. Secondly, a marked degree of auto-agglutination comparable to that occuring in trypanosomiasis has not been described in any other of the diseases in which anaemia is a distinctive feature. Dudgeon* examined the blood of twenty-six cases of anaemia due to various causes without finding a single example of auto-agglutination. It is doubtful, however, whether the technique adopted by Dudgeon is suitable for the recognition of small amounts of auto-agglutinin. Then, again, it is generally recognised as impossible to evoke the production of auto-bodies experimentally by inoculating an animal with its own tissues.

Experiment.—A rabbit was injected intraperitoneally with 10 c.c. of its own erythrocytes which had been laked with distilled water and the resulting solution made isotonic with sodium chloride.

* 'On the Presence of Haemagglutinins, etc., in the Blood obtained from Infectious and Non-infectious Diseases in Man,' Roy. Soc. Proc., 1909, B, Vol. LXXX, p. 531.

This injection was repeated after an interval of a week. No increase of auto-agglutinin was found to occur in the animal's blood.

Another possible explanation for the cause of the development of auto-agglutinin is that it is formed by the animal mechanism as a direct response to the stimulus of the pathogenic agent. In the consideration of this question it is necessary to inquire whether auto-agglutinin alone is present in excess in infected blood or whether we have at the same time a corresponding alteration in the iso- and hetero-agglutinin contents of the serum.

Mention has already been made of the fact that in every case where the serum of an infected animal was found to possess the property of clumping its own erythrocytes to a considerable degree, it also agglutinated markedly those of other members of the same species.

It was further observed that the plasma of infected animals frequently appeared to agglutinate the red cells of animals belonging to different species to a greater extent than normal. The case of human trypanosomiasis already referred to presented an excellent example of an increased capacity on the part of an infected serum to agglutinate foreign erythrocytes. The serum of this case clumped the red corpuscles of rats, guinea-pigs and rabbits in a remarkable manner. A few drops of the inactivated serum when added to an equal volume of the blood of one of these animals caused intense agglutination in a few seconds at room temperature. The action of normal human sera on these corpuscles was much slower and did not approach that of the former in intensity.

Experiments were undertaken with the object of comparing quantitatively the auto-, iso-, and hetero-agglutinin in the blood of several infected animals with that existing in the blood of normal animals of the same kind.

Technique.—The method adopted was that previously used for comparing the amount of auto- and iso-agglutinin in the defibrinated plasma separated from the red cells at 37° C. with that present in the serum obtained from blood which had clotted at 0° C.

Inactivated defibrinated plasma was prepared from normal

Rabbit 1 and from Rabbit 896 (infected with *T. dimorphon*), and a 5 per cent. suspension of washed erythrocytes from the following animals: normal Rabbits 1 and 2, Rabbit 896 and a normal horse and guinea-pig.

From the results of this experiment, details of which are given in Table IV, and from other observations of the same kind one is led to conclude that in the blood of infected animals, in addition to an excess of auto-agglutinin, there is also frequently a corresponding increase in iso- and hetero-agglutinin.

The question now arises as to whether these reactions are manifestations of the same body or of different specific agglutinins. The procedure usually adopted for the solution of problems of this nature is the saturation of a portion of the serum by the red cells of one of the varieties in question, and then, after allowing reaction to take place for some hours, centrifugalising and examining the extracted serum regarding its agglutinating action on the red cells of the kind used for extraction and also on the other varieties of erythrocytes.

Malkoff,* adopting this technique, arrived at the conclusion that there exist in goat's serum, which is capable of agglutinating the erythrocytes of many kinds of animals, different specific agglutinins, each of which has a specific affinity for the corresponding variety of red cells.

Experiment.—(1) One volume of defibrinated plasma of Rabbit 896 (infected with *T. dimorphon*) was extracted for twelve hours at 0° C. with an equal volume of the undiluted red blood cells of the same animal. (Extracted plasma A.) (2) Here the proportion of plasma to erythrocytes was one to five. (Extracted plasma B.) (3) One volume of the same plasma was treated with one volume of normal horse's red cells. (Extracted plasma C.)

A 5 per cent. suspension of washed erythrocytes was prepared from the following animals: Rabbit 896, normal Rabbit 1, normal Donkeys 1 and 2, a normal guinea-pig, horse, *Macacus rhesus*, *Cercopithecus callitrichus*, and human being.

The results indicate that complete extraction of the infected plasma of Rabbit 896 by its own erythrocytes and those of a normal

* 'Beiträge zur Frage der Agglutination von rother Blutkörperchen,' Deutsche Med. Woch., 1900, No. 14.

TABLE IV.—Comparison of the Amount of Auto-, Iso-, and Hetero-agglutinin in the Plasma of Rabbit 896 (infected with *T. dimorphon*) and Normal Rabbit 1

Equal volumes of red cell suspension and diluted plasma used. Temperature of experiment 0° C.		
5 per cent. suspension of washed erythrocytes in normal saline solution	Plasma of Rabbits 1 and 896 diluted with increasing amounts of normal saline solution	Result
Rabbit 1	Rabbit 1 undiluted	Complete agglutination in 20 mins.
,, 1	,, 896 ,, ...	,, ,, 20 ,,
,, 2	,, 1 ,, ...	,, ,, 20 ,,
,, 2	,, 896 ,, ...	,, ,, 20 ,,
,, 896	,, 1 ,, ...	,, ,, 20 ,,
,, 896	,, 896 ,, ...	,, ,, 20 ,,
Horse	,, 1 ,, ...	,, ,, 10 ,,
	,, 896 ,, ...	,, ,, 10 ,,
Guinea-pig	,, 1 ,, ...	Marked ,, 45 ,,
	,, 896 ,, ...	Complete ,, 45 ,,
Rabbit 1	,, 1 diluted with twice its vol. of 0·9 per cent. NaCl solution	Nil ,, 45 ,,
,, 1	,, 896 ,,	Complete ,, 20 ,,
,, 2	,, 1 ,,	Slight ,, 20 ,,
,, 2	,, 896 ,,	Complete ,, 20 ,,
,, 896	,, 1 ,,	Nil ,, 20 ,,
,, 896	,, 896 ,,	Complete ,, 20 ,,
Horse	,, 1 ,,	Marked ,, 45 ,,
	,, 896 ,,	Complete ,, 15 ,,
Guinea-pig	,, 1 ,,	Nil ,, 45 ,,
	,, 896 ,,	Complete ,, 15 ,,
Rabbit 1	,, 1 diluted with 4 times its vol. of 0·9 per cent. NaCl solution	Nil ,, 15 ,,
,, 1	,, 896 ,,	Complete ,, 15 ,,
,, 2	,, 1 ,,	Nil ,, 15 ,,
,, 2	,, 896 ,,	Complete ,, 30 ,,
,, 896	,, 1 ,,	Nil ,, 30 ,,
,, 896	,, 896 ,,	Complete ,, 30 ,,
Horse	,, 1 ,,	Slight ,, 30 ,,
	,, 896 ,,	Complete ,, 30 ,,
Guinea-pig	,, 1 ,,	Nil ,, 30 ,,
	,, 896 ,,	Complete ,, 20 ,,
Rabbit 1	,, 896 diluted with 6 times its vol. of 0·9 per cent. NaCl solution	,, ,, 20 ,,
,, 2	,, 896 ,,	,, ,, 30 ,,
,, 896	,, 896 ,,	,, ,, 30 ,,
Horse	,, 896 ,,	Marked ,, 45 ,,
Guinea-pig	,, 896 ,,	Complete ,, 15 ,,
Rabbit 1	,, 896 diluted with 9 times its vol. of 0·9 per cent. NaCl solution	,, ,, 20 ,,
,, 2	,, 896 ,,	Partial ,, 45 ,,
,, 896	,, 896 ,,	Complete ,, 30 ,,
Horse	,, 896 ,,	Trace ,, 45 ,,
Guinea-pig	,, 896 ,,	Complete ,, 15 ,,
Rabbit 1	,, 896 diluted with 14 times its vol. of 0·9 per cent. NaCl solution	Slight ,, 45 ,,
,, 2	,, 896 ,,	Nil ,, 45 ,,
,, 896	,, 896 ,,	Slight ,, 45 ,,
Horse	,, 896 ,,	Nil ,, 45 ,,
Guinea-pig	,, 896 ,,	Slight ,, 45 ,,

horse does not completely destroy the agglutinating action of the plasma on the red blood cells of other animals, although it is to be noted that in most cases when the plasma had been extracted with five times its volume of its own red cells there was a marked lessening or even total disappearance of this action. This diminution of the agglutinating action of the plasma cannot be explained by mere dilution with the small amount of saline solution adhering to the red cells, as the plasma still caused marked agglutination after the addition of fifteen times its volume of 0·9 per cent. sodium chloride solution.

It is doubtful, however, whether experiments of this kind really have the importance that has been assigned to them by Malkoff and others.

Landsteiner and Sturli* using normal horse and dog serum and eleven varieties of erythrocytes, confirmed Malkoff's observation that saturation of the serum with one kind of red blood cell deprived it of the power to agglutinate this variety, and this only. They furthermore showed that red cells which had already been once completely agglutinated were still able to react with another kind of serum, and that the new serum after the reaction had lost its power to agglutinate fresh corpuscles of the same kind. Hence, as Landsteiner points out, the problem had assumed a very complex aspect, the enormous number of specific agglutinins in normal serum appearing uneconomic.

Landsteiner and Sturli suggest another hypothesis to explain these facts, namely, that during the process of agglutination some substance passes from the red cell to the serum, and that after complete agglutination the serum, in consequence of the combination, agglutinin + corpuscle substance, can no longer react with red cells of the same kind, but can with those of other animals. By this theory they maintain that the facts can be explained without the necessity for assuming the presence of an enormous number of differently acting substances or groups of substances in normal serum.

A certain amount of support is afforded this view by the observation of Landsteiner that a watery extract of the corpuscles of a turkey, when added to horse serum, almost completely prevents its agglutinating action on the red cells of the turkey, but only in

* 'Ueber die Hamagglutinine normaler Sera,' Wien. Klin. Woch., 1902, p. 38.

TABLE V.—Agglutinating Action of Plasma which had been previously Extracted with Red Blood Cells

Equal volumes of red cell suspension and plasma used. Temp. of experiment 0° C.		
5 per cent. suspension of washed erythrocytes in normal saline solution	Untreated plasma of Rabbit 896 (infected with *T. dimorphon*) and also plasma of the same animal extracted with red cells as follows: A, with an equal volume of red blood cells of Rabbit 896 B, with five times its volume of red cells of Rabbit 896. C, with an equal volume of red cells of a normal horse	Result
Rabbit 896	Untreated plasma	Complete agglutination in 10 mins.
	Extracted ,, A	Partial ,, 45 ,,
	,, ,, B	No ,, 45 ,,
	,, ,, C	Complete ,, 15 ,,
Rabbit 1	Untreated plasma	Complete ,, 15 ,,
	Extracted ,, A	,, ,, 30 ,,
	,, ,, B	No ,, 45 ,,
	,, ,, C	Complete ,, 15 ,,
Donkey 1	Untreated plasma	Complete ,, 15 ,,
	Extracted ,, A	Almost complete ,, 45 ,,
	,, ,, B	No ,, 45 ,,
	,, ,, C	Marked ,, 45 ,,
Donkey 2	Untreated plasma	Complete ,, 15 ,,
	Extracted ,, A	,, ,, 30 ,,
	,, ,, B	Trace ,, 45 ,,
	,, ,, C	Complete ,, 45 ,,
Guinea-pig	Untreated plasma	Complete ,, 15 ,,
	Extracted ,, A	,, ,, 15 ,,
	,, ,, B	Partial ,, 45 ,,
	,, ,, C	Complete ,, 15 ,,
Horse	Untreated plasma	Complete ,, 10 ,,
	Extracted ,, A	,, ,, 15 ,,
	,, ,, B	Slight ,, 45 ,,
	,, ,, C	Partial ,, 45 ,,
Macacus rhesus	Untreated plasma	Complete ,, 15 ,,
	Extracted ,, A	,, ,, 30 ,,
	,, ,, B	Slight ,, 45 ,,
	,, ,, C	Complete ,, 30 ,,
Cercopithecus callitrichus ...	Untreated plasma	Complete ,, 15 ,,
	Extracted ,, A	,, ,, 15 ,,
	,, ,, B	,, ,, 30 ,,
	,, ,, C	,, ,, 15 ,,
Human being	Untreated plasma	Complete ,, 10 ,,
	Extracted ,, A	,, ,, 20 ,,
	,, ,, B	Marked ,, 45 ,,
	,, ,, C	Complete ,, 15 ,,

a very slight degree on other kinds of blood. This last observation was subsequently confirmed by Lazar.* However, unless all traces of the stromata of the red cells had been removed from the haemoglobin solution—and this is by no means an easy performance —an obvious explanation for this inhibiting action of such solutions would be that the stromata themselves had fixed the agglutinins present in the horse serum, and consequently there would be little, if any, left to act upon the red corpuscles. Naturally, in this case, the inhibiting action would be specific for the variety of red cells from which the haemoglobin solution was made.

The fact that the phenomenon of auto-agglutination is reversible allows one to approach the subject of specificity from a different point of view, namely, by extracting the completely agglutinated red cells with a small quantity of normal saline solution at 37° C., and then investigating the nature of the digest.

Experiment.—To 10 c.c. of defibrinated plasma of Rabbit 1035 (infected with *T. brucei*) were added 0·2 c.c. of the red cells of the same animal. After allowing the mixture to stand in the ice chest for twelve hours with occasional stirrings the supernatant plasma was decanted off, and the clumped red blood cells washed four times with at least ten times their volume of normal saline solution at 0° C.; 0·2 c.c. of normal salt solution was then added to the agglutinated mass of red cells and the mixture allowed to digest at 40° C. for half an hour. At the end of this time no trace of agglutination was visible. The red cells were then quickly thrown down by centrifugalisation and the supernatant fluid removed (Digest solution).

A 5 per cent. suspension of red blood cells in normal saline solution was prepared from the following animals: Rabbit 1035 (infected with *T. brucei*), normal Rabbit A, Donkey 2 (infected with *T. rhodesiense*), normal Donkey A, Goat 1041 (infected with *T. rhodesiense*), normal Goat A, and from a normal rat, guinea-pig, dog, horse, *Macacus rhesus*, *Cercopithecus callitrichus*, and human being.

The capacity of the untreated plasma of Rabbit 1035 and of the solution prepared by digesting the agglutinated red cells with normal saline at 0° C. to agglutinate these different erythrocytes was then examined.

* 'Ueber die Bedeutung der lipoiden Stoffe der rothen Blutkörperchen für den Mechanismus der Agglutination,' Wien. Klin. Woch., 1905, p. 1012.

TABLE VI.—Agglutinating Action of a Solution obtained by Digesting Auto-agglutinated Red Blood Cells with Normal Saline Solution at 40° C.

Equal volumes of red cell suspension and defibrinated plasma or digest solution used. Temperature of experiment 0° C.		Result
5 per cent. suspension of washed erythrocytes in normal saline solution	Untreated defibrinated plasma of Rabbit 1035 (infected with nagana), and solution obtained by digesting the auto-agglutinated red cells of the same animal with normal saline solution at 37° C.	
Rabbit 1035 (infected with *T. brucei*)	Defibrinated plasma	Complete agglutination in 10 mins.
	Digest solution	,, ,, 10 ,,
Normal Rabbit A	Defibrinated plasma	,, ,, 10 ,,
	Digest solution	,, ,, 10 ,,
Donkey 2 (infected with *T. rhodesiense*)	Defibrinated plasma	,, ,, 10 ,,
	Digest solution	,, ,, 30 ,,
Normal Donkey A ...	Defibrinated plasma	,, ,, 15 ,,
	Digest solution	Slight ,, 45 ,,
Goat 1041 (infected with *T. rhodesiense*)	Defibrinated plasma	Complete ,, 10 ,,
	Digest solution	No ,, 45 ,,
Normal Goat A	Defibrinated plasma	Complete ,, 10 ,,
	Digest solution	No ,, 45 ,,
Rat	Defibrinated plasma	Complete ,, 15 ,,
	Digest solution	,, ,, 20 ,,
Guinea-pig	Defibrinated plasma	,, ,, 15 ,,
	Digest solution	,, ,, 20 ,,
Dog	Defibrinated plasma	,, ,, 5 ,,
	Digest solution	,, ,, 10 ,,
Horse	Defibrinated plasma	,, ,, 10 ,,
	Digest solution	Partial ,, 45 ,,
Macacus rhesus	Defibrinated plasma	Complete ,, 15 ,,
	Digest solution	,, ,, 30 ,,
Cercopithecus callitrichus ...	Defibrinated plasma	,, ,, 15 ,,
	Digest solution	,, ,, 15 ,,
Human being	Defibrinated plasma	,, ,, 10 ,,
	Digest solution	,, ,, 15 ,,

N.B.—The four specimens of normal saline solution which had been used for washing the clumped red cells were also examined. With the exception of occasional traces in the first, no agglutinin was found in these solutions.

The information obtained from observations of this kind is extremely interesting. In the experiment recorded a substance was extracted from the auto-agglutinated erythrocytes of a rabbit infected with *T. brucei* which clumped not only its own erythrocytes and those of other rabbits, but also the red cells of many other animals of different species. In other words it would appear that the auto-agglutinin is not a body equipped with a high degree of

specificity, but that it can also act as iso- and hetero-agglutinin on the erythrocytes of other rabbits and those of animals of widely different species.

VALUE OF THE PHENOMENON AS A DIAGNOSTIC SIGN

Before discussing this question it is necessary to emphasise the importance of careful observation in determining whether a certain blood really agglutinates or not. So far as can be gathered from the papers in which the existence of the phenomenon in trypanosomiasis has been recorded, it has been invariably decided from the examination of cover-slip preparations of the blood. Although a considerable degree of auto-agglutination is easily recognised in a well-made cover-slip preparation, yet it is often extremely difficult, or even impossible, to decide whether the red cells are really agglutinated when the phenomenon is not so distinct. A certain amount of massing together of the erythrocytes is frequently evident at the edges of even the best cover-slip preparations of normal blood, whereas if the slide and cover-slip be not perfectly clean the red cells are found to be anything but evenly distributed, but are grouped together into little masses and rouleaux, separated from one another by plasma—an appearance closely resembling that to be observed in infected blood when the amount of auto-agglutination is slight. On the other hand, a slight degree of auto-agglutination can be easily obscured by pressure on the cover-slip resulting in the separation of the erythrocytes one from the other.

Furthermore, it has been shown that small amounts of auto-agglutinin exist constantly in the blood of many normal animals. In horses and donkeys auto-agglutinin is sometimes present in such an extent as to give rise to a more or less characteristic appearance in cover-slip preparations. This is specially the case when the preparations are made out of doors at a somewhat low temperature. However, I have never observed in cover-slip preparations of the blood of normal animals a condition approaching in intensity the well-marked clumping obtaining in infected cases. When a high degree of auto-agglutination exists the corpuscles are seen to have become agglomerated into tight clumps, the outlines of the individual cells being indistinct, or even

completely lost, so that the clumps appear to consist of red cells which have fused together into a homogeneous mass. In order to evoke as characteristic an appearance as possible the preparation should be made at the lowest temperature practicable.

The next point to be considered is whether auto-agglutination is a constant feature in trypanosomal infections.

Martin, Lebœuf, and Roubaud* stated that in the large number of cases of human trypanosomiasis examined by them in the French Congo, auto-agglutination was always present. In the tables appearing in their report, the condition of the blood as regards auto-agglutination is indicated by numbers from 0 to 10, the cipher meaning that there is no agglutination, whereas the greatest degree of agglutination is indicated as 10; the intermediate figures denote intermediate degrees of agglutination.

In view of the technique used by them—the mere examinations of cover-slip preparations of the fresh blood—such a classification appears to be a somewhat unwarrantable refinement.

Todd† in a recent paper classifies as regards auto-agglutination a large number (1406) of cases examined by Dutton and himself in the Congo Free State. Of the 395 cases in which auto-agglutination was present, trypanosomes were found in only 183. However, as Todd himself states, probably because of the insufficient search for them (the cases were seen and examined on one occasion only), trypanosomes were present much more often than they were found.

Later in the same paper it is stated that only in three cases were trypanosomes not present when an extremely well-marked auto-agglutination was recorded. One of these was a case of relapsing fever; another was a much emaciated marasmic individual, and the third was a case of syphilis.

Regarding the frequency of the phenomenon in the blood of experimentally infected animals, it need only be stated that as a rule auto-agglutination is best marked in the blood of the larger animals, *e.g.*, horse and donkey. It is usually also very distinct in the monkey, dog, rabbit, and goat. In the rat, mouse, and guinea-pig it is generally slight or absent.

* Rapport de la Mission d'Études de la Maladie du Sommeil au Congo Française, 1906-8, p. 281.

† 'A Note on the Occurrence of Auto-agglutination of the Red Cells in Human Trypanosomiasis,' Bull. Soc. Path. Exot., 1910, p. 438.

In the ordinary course of events the infected animal shows parasites in its blood for some time before a distinct auto-agglutination develops. Occasionally, however, auto-agglutination appears before trypanosomes have been found in the blood. I have had under observation a goat, infected with *T. gambiense*, in which trypanosomes have never been seen in the blood, but in which a well-marked auto-agglutination had developed. The blood was shown to be infective by injection into rats.

The last point to be decided in considering the value of the phenomenon as a diagnostic sign is whether it occurs in other diseases besides trypanosomiasis. In addition to the three cases mentioned by Todd, auto-agglutination has occasionally been noted in persons suffering from other diseases than sleeping sickness.

Klein,* in 1890, found auto-agglutination of the red blood cells in a case of hepatic cirrhosis (Hanot). Dudgeon† mentions the case of a West Indian negro which was considered to be one of tertiary hepatic syphilis, where the blood exhibited spontaneous clumping. He also found auto-agglutinin in the blood of a case of long standing epilepsy. Martin and Darré‡ assert that the phenomenon is to be met with in certain forms of icterus due to haemolysis.

Quite recently Nattan-Larrier§ described the existence of auto-agglutination in rats infected with the *Spirilla obermierei*. It is interesting to note in this connection that of Todd's three cases which exhibited well marked auto-agglutination, but were not infected with trypanosomes, one was a case of relapsing fever and another a case of syphilis, as was also the only definite instance of the phenomenon seen by Dudgeon. It is of importance to know whether auto-agglutination is often present in spirochaetal infections.

In conclusion it may be stated that in the light of the information obtainable a well-marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence, apart from infection with trypanosomes.

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* 'Ueber die Untersuchung der Formelemente des Blutes und ihre Bedeutung für die praktische Medicin,' Wien. Klin. Woch., 1890, Nos. 36-40.

† *Loc. cit.*

‡ Bull. et Mémoires Soc. Méd. des Hôpitaux de Paris, 1909, p. 599.

§ 'L'Autoagglutination des Hématies dans la Spirillose Expérimentale,' Bull. Soc. Path. Exot. 1910, p. 425.

SUMMARY

Auto- and iso-agglutinin are present in the blood of cases of sleeping sickness and of animals infected with trypanosomiasis.

Reaction between auto-agglutinin and red blood cells takes place only at low temperatures.

Auto-agglutinin can be removed from plasma by absorption with the erythrocytes of the same animal at 0° C.

The reaction between auto-agglutinin and red blood cells is reversible.

Auto-agglutinin exists in small amounts in the blood of many normal animals.

Auto-, iso- and hetero-agglutinin are frequently present in much greater amount in the blood of infected animals than in that of normal animals, and it is due to this fact that clumping of the red blood cells is often visible in fresh cover-slip preparations of the blood of infected animals.

From the red blood cells of an infected animal which have been agglutinated in the cold by the plasma of the same animal an active substance can be extracted with normal saline solution at 37° C.

This substance agglutinates not only the red cells of the same animal and other members of the same species, but also those of many animals of different species.

It is to be inferred from the information at present available that a marked degree of auto-agglutination of the red blood cells is an extremely rare occurrence apart from an infection with trypanosomes.

ON A NEW GENUS OF CULICINAE FROM THE AMAZON REGION

BY
R. NEWSTEAD, M.Sc., &c.,
AND
H. F. CARTER.

(*Received for publication* 15 *February*, 1911)

Thomasina, nov. gen. (Newstead and Carter)

Palpi of the male (fig. 1) much shorter than the proboscis; they are also rather slender and composed of four segments, of

FIG. 1. Head of ♂ *Thomasina longipalpis*. (Newstead and Thomas.)

which the terminal one is very short and narrower than the preceding one. *Antennae* (fig. 1) rather sparsely clothed with

hairs. *Legs* with long and somewhat outstanding scales at the apex of the femora; the fourth and fifth segments of the front and middle tarsi very short and broad, being compressed laterally.

Scale structure of both sexes. Those of the head and thorax as in *Mansonia*, with the exception of a few broad curved ones just in front of the scutellum and at the base of the wings; pleurae densely clothed with large, broad, spindle-shaped scales; prothoracic lobes distally clothed with spindle-shaped ones; scutellum with long falciform scales. *Wings* with *Mansonia*-like scales, the outstanding ones being, however, much longer.

Type, *Thomasina longipalpis* (Newstead and Thomas).

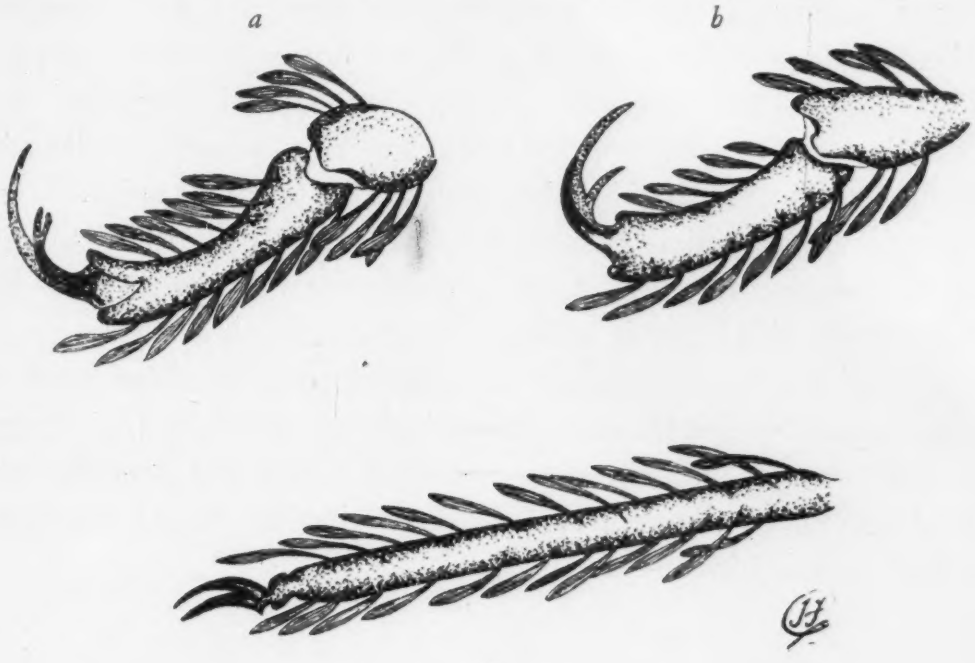
FIG. 2. Palpus of ♀ *Thomasina longipalpis*. (Newstead and Thomas.)

In describing the female of this species, Newstead and Thomas* referred it to the genus *Mansonia* owing to a general resemblance which the scale structure bears to this genus; and, although these authors had noted the peculiar character of the palpi, they did not think this was of sufficient importance to warrant the erection of a new genus for the reception of this insect.

* Ann. of Trop. Med. and Parasit., Vol. IV, No. 1, p. 145.

Having recently received several examples of both sexes, it was at once seen that the morphological characters of the palpi and tarsi were so markedly different from those of *Mansonia* that the species could no longer remain in that genus.

The marked characteristics of *Thomasina* are that the *palpi of the male are short, while those of the female are relatively long* (figs. 1 and 2), the latter almost one-third the length of the proboscis; and that *the fourth tarsal segment of the fore and mid-legs, in both sexes, is very small and flattened* (fig. 3, *a*, *b*).



a *b* *c*

FIG 3. *a* and *b*. Fourth and fifth tarsal segments and ungues of the fore and mid legs of ♂ *Thomasina longipalpis*. (Newstead and Thomas.)

c. Fifth segment and ungues of the same.

We have much pleasure in dedicating this insect to our colleague, Dr. H. Wolferstan Thomas, who has contributed so much towards our knowledge of the mosquitos of the Amazon region at Manaos and elsewhere.

Thomasina longipalpis (Newstead and Thomas) (1910).
Mansonia longipalpis (♀ only) (Newstead and Thomas) (1910)*.

MALE.—Colour and general characteristics as in the female. *Palpi* clothed with black, white, and ochreous-yellow scales, the

* Ann. of Trop. Med. and Parasit., Vol. IV, No. 1, p. 145.

latter predominating; the articulations, the apical segment, and base of the first segment, white scaled. *Proboscis* very thick, slightly stouter than that of the female, but as far as can be seen, with the same distribution of scales. *Legs* (fig. 3), fourth tarsal segments of the first pair of legs slightly broader than long, in the ♀ larger and considerably longer than broad; ungues unequal, the larger with a distinct sub-median tooth, the smaller simple. Fourth segments of the second pair of legs longer and narrower than in the first pair, in the ♀ shorter and slightly broader and about the same size as the corresponding ones of the ♂; ungues as in the fore legs. Tarsi of the hind legs normal in both sexes, the ungues equal and simple. *Genitalia*:—Basal segment stout, gradually tapering to a rounded apex; superior clasper slender, about half the length of the basal lobe, with a long terminal spine; inferior clasper forming a fairly distinct lobe bearing a pair of thick spines. Harpes and harpagones stout, the former terminating in three distinct teeth, the latter in a single large one.

In addition to the female characters given by Newstead and Thomas, and besides those mentioned above, there are two others of some importance, viz., the ungues are all equal and simple, and in several specimens the ventral surface of the last tarsal segment of the hind legs is black scaled.

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